CA Search
2/15/99
08/860,844
CNIKID, AM

L5 ANSWER 1 OF 32 CA COPYRIGHT 1999 ACS
AN 124:166374 CA
TI Cleavage of double-stranded DNA by 'metalloporphyrin-linker-oligonucleotide' molecules: influence of the linker
AU Bigey, Pascal; Pratviel, Genevieve; Meunier, Bernard
CS Lab. Chimie Coordination CNRS, Toulouse, 31077, Fr.
SO Nucleic Acids Res. (1995), 23(19), 3894-900
CODEN: NARHAD; ISSN: 0305-1048
DT Journal
LA English
AB Manganese porphyrin-linker- ***triple*** - ***helix*** -forming oligonucleotide mols. were prepd. and their ability to cleave in vitro a double-stranded DNA target present in the ***HIV*** -1 genome was studied. The nature of the linker is a detg. factor of the cleavage efficiency. Cleavage yields as high as 80% were obsd. when the linker was a spermine residue and in the absence of a large excess of free spermine known to stabilize ***triplex*** structures. The hydrophobic nature of aliph. diamine linker modified the cleaver-DNA interactions and reduced the efficiency of DNA cleavage.

L5 ANSWER 2 OF 32 CA COPYRIGHT 1999 ACS
AN 124:81693 CA
TI Inhibition of ***HIV*** -1 reverse transcription by ***triple*** - ***helix*** forming oligonucleotides with viral RNA. [Erratam to document cited in CA122:310452]
AU Volkmann, S.; Jendis, J.; Frauendorf, A.; Moelling, K.
CS Max-Planck-Inst. Mol. Genetik Abt.Schuster, Berlin, D-14195, Germany
SO Nucleic Acids Res. (1995), 23(18), 3804
CODEN: NARHAD; ISSN: 0305-1048
DT Journal
LA English
AB The errors were not reflected in the abstr. or the index entries.

L5 ANSWER 3 OF 32 CA COPYRIGHT 1999 ACS
AN 124:47360 CA
TI The high stability of the .***triple*** ***helixes*** formed between short purine oligonucleotides and SIV/ ***HIV*** -2 vpx genes is determined by the targeted DNA structure
AU Svinarchuk, Fedor; Monnot, Monique; Merle, Aurelie; Malvy, Claude; Fermandjian, Serge
CS Lab. Biochim.-Enzymol., Inst. Gustave Roussy, Villejuif, 94805, Fr.
SO Nucleic Acids Res. (1995), 23(19), 3831-6
CODEN: NARHAD; ISSN: 0305-1048
DT Journal
LA English

AB In our previous works we have shown that the oligonucleotides 5'-GGGGAGGGGGAGG-3' and 5'-GGAGGGGGAGGGG-3' give very stable and specific triplexes with their target double stranded DNAs. The target for the invariable part of these oligonucleotides, 5'-GGAGGGGGAGG-3', is found in a highly conserved 20 bp long purine/pyrimidine tract of the vpx gene of the SIV and ***HIV*** -2 viruses and could be a target for oligonucleotide directed antivirus therapy. Here we report on the ability of four purine oligonucleotides with different lengths (11-, 14-, 17- and 20-mer) to form triplexes with the purine/pyrimidine stretch of the vpx gene. ***Triplex*** formation was tested by joint di-Me sulfate (DMS) footprint, gel-retardation assay, CD (CD) and UV-melting studies. Di-Me sulfate footprint studies revealed the antiparallel orientation of the third strand to the purine strand of the Watson-Crick duplex. However, the protection of the guanines at the ends of the target sequence decreased as the length of the third strand oligonucleotide increased. Melting temp. studies provided profiles with only one transition for all of the triplexes. The melting temps. of the triplexes were found to be the same as for the targeted duplex in the case of the 11- and 14-mer third strands while for the 17- and 20-mer third strands the melting temp. of the triplexes were correspondingly 4 and 8.degree.C higher than for the duplex. Heating and cooling melting curves were reversible for all of the tested triplexes except one with the 20-mer third strand oligonucleotide. CD spectra showed the ability of the target DNA to adopt an A-like DNA conformation. Upon ***triplex*** formation the A-DNA form becomes even more pronounced. This effect depends on the length of the third strand oligonucleotide: the CD spectrum shows a 'classical' A-DNA shape with the 20-mer. This is not obsd. with the purine/pyrimidine stretch of the ***HIV*** -1 DNA which keeps a B-like spectrum even after ***triplex*** formation. We suggest, that an A-like duplex DNA is required for the formation of a stable DNA purine(purine-pyrimidine) ***triplex*** .

L5 ANSWER 4 OF 32 CA COPYRIGHT 1999 ACS
AN 123:278052 CA
TI DNA encoding ***triple*** ***helix*** -forming RNA for control of gene expression
IN Giovannangeli, Carine; Helene, Claude
PA Centre National de la Recherche Scientifique, Fr.; Museum National d'Histoire Naturelle; Institut National de la Sante et de la Recherche Medicale (INSERM)
SO Fr. Demande, 24 pp.
CODEN: FRXXBL
PI FR 2714383 A1 19950630
AI FR 93-15798 19931229
DT Patent

LA French
AB DNA encoding RNA capable of forming a ***triple*** ***helix*** with a target single-stranded DNA and the RNA itself, expression vectors contg. said DNA, pharmaceutical compns. contg. the DNA or expression vector, and a method for regulation of gene expression in a cell using the DNA or expression vector are claimed. The RNA comprises a section capable of forming a double helix with the target DNA and a section capable of forming a ***triple*** ***helix*** with the double helix. Many DNA sequences encoding RNA which may form ***triple*** ***helixes*** with ***HIV*** nucleic acid or with insulin-like growth factor I gene were presented.

L5 ANSWER 5 OF 32 CA COPYRIGHT 1999 ACS
AN 123:17943 CA
TI Therapeutic delivery compositions containing compounds capable of altering nucleic acid sequence function and surfactants
IN Emanuele, R. Martin; Allaudeen, Hameedsulthan S.; Kousoulas, Konstantin G.
PA Cytrx Corp., USA
SO PCT Int. Appl., 35 pp.
CODEN: PIXXD2
PI WO 9510265 A1 19950420
DS W: AU, CA, JP, KR
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
AI WO 94-US11594 19941012
PRAI US 93-138271 19931015
DT Patent
LA English
AB The present invention relates to compns. and methods for treating infectious diseases and genetic disorders through gene therapy and intracellular delivery of antisense oligonucleotides or other nucleic acid sequences. The compns. comprise an administerable admix. of an effective amt. of a therapeutic compd. capable of altering nucleic acid sequence function and an effective amt. of a surface active nonionic block copolymer having the following general formula: HO(C2H4O)b(C3H6O)a(C2H4O)bH wherein a is an integer such that the hydrophobe represented by (C3H6O) has a mol. wt. 750-15,000 and b is an integer such that the hydrophile portion represented by (C2H4O) constitutes 1-50% of the compd. An antisense oligonucleotide to ***HIV*** art/trs genes was mixed with polyoxyethylene-polyoxypropylene block copolymer and i.v. administered to a patient infected with ***HIV*** virus.

L5 ANSWER 6 OF 32 CA COPYRIGHT 1999 ACS
AN 122:310452 CA
TI Inhibition of ***HIV*** -1 reverse transcription by ***triple*** -

helix forming oligonucleotides with viral RNA
AU Volkmann, Silke; Jendis, Joerg; Frauendorf, Albrecht; Moelling, Karin
CS Max-Planck-Inst. Mol. Genetik Abt. Schuster, Berlin, D-14195, Germany
SO Nucleic Acids Res. (1995), 23(7), 1204-12
CODEN: NARHAD; ISSN: 0305-1048
DT Journal
LA English
AB Reverse transcription of retroviral RNA into double-stranded DNA is catalyzed by reverse transcriptase (RT). A highly conserved polypurine tract (PPT) on the viral RNA serves as primer for plus-strand DNA synthesis and is a possible target for ***triple*** - ***helix*** formation. ***Triple*** - ***helix*** formation during reverse transcription involves either single-stranded RNA or an RNA.cntdot.DNA hybrid and ***triplex*** -forming oligonucleotides (TFOs) consisting either of DNA or RNA. Three strand ***triple*** - ***helixes*** inhibit RNase H cleavage of the PPT-RNA.cntdot.DNA hybrid and initiation of plus-strand DNA synthesis in vitro. ***Triple*** - ***helix*** formation on a single-stranded RNA target has also been tested in a two-strand-system with TFOs prevent RNase H cleavage of the PPT-RNA and initiation of plus-strand DNA synthesis in vitro. In cell culture expts. one TFO is an efficient inhibitor of retrovirus replication, leading to a block of p24 synthesis and inhibition of syncytia formation in newly infected cells.

L5 ANSWER 7 OF 32 CA COPYRIGHT 1999 ACS
AN 122:100618 CA
TI Sequence-specific DNA double-strand breaks induced by ***triplex*** forming 125I labeled oligonucleotides
AU Panyutin, Igor G.; Neumann, Ronald D.
CS Dep. Nuclear Med., Natl. Institutes Health, Bethesda, MD, 20892, USA
SO Nucleic Acids Res. (1994), 22(23), 4979-82
CODEN: NARHAD; ISSN: 0305-1048
DT Journal
LA English
AB A ***triplex*** -forming oligonucleotide (TFO) complementary to the polypurine-polypyrimidine region of the nef gene of the Human Immunodeficiency Virus (***HIV***) was labeled with 125I at the C5 position of a single deoxycytosine residue. Labeled TFO was incubated with a plasmid contg. a fragment of the nef gene. Decay of 125I was found to cause double-strand breaks (DSB) within the nef gene upon ***triplex*** formation in a sequence specific manner. No DSB were detected after incubation at ionic conditions preventing ***triplex*** formation or when TFO was labeled with 32P instead of 125I. Mapping DSB sites with single base resoln. showed that they are distributed within 10 bp of a max. located exactly opposite the position of the [125I] IdC in

the TFO. We est. that on av. the amt. of DSB produced per decay is close to one.

L5 ANSWER 8 OF 32 CA COPYRIGHT 1999 ACS
AN 122:48463 CA
TI Combinatorial oligomer-binding screening assays for transcription factors and elements and other biomolecules
IN Ecker, David J.; Vickers, Timothy A.; Davis, Peter W.
PA ISIS Pharmaceuticals, Inc., USA
SO PCT Int. Appl., 106 pp.
CODEN: PIXXD2
PI WO 9421825 A1 19940929
DS W: CA, JP
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
AI WO 94-US2166 19940301
PRAI US 93-32852 19930316
DT Patent
LA English
AB Methods for identifying oligomers that specifically bind a transcription factor or other target mol. in a pool of primarily randomly assembled subunits are described. The method involves repeated syntheses of increasingly simplified sets of oligomers coupled with novel selection procedures to identify the oligomers with the highest activity. The method does not require the enzymic synthesis of the oligomer ligands and so it can be used with any set of monomers that can be oligomerized in a controlled fashion. The synthesis of pools of modified oligonucleotides and the screening of these pools to identify ligands for regulatory elements of animal viruses is demonstrated. Pools typically started with sub-pools 65,536 members with each of the sub-pools having at least one base fixed. These sub-pools were screened for binding to the target and a second pool with a second fixed base prepd. (with a lower complexity) and so on.

L5 ANSWER 9 OF 32 CA COPYRIGHT 1999 ACS
AN 121:273865 CA
TI Oligonucleotides forming ***triple*** ***helixes*** with non-B purine-rich DNA for regulation of gene expression
IN Yoon, Kyonggeun; Lu, Meiqing
PA Apollon, Inc., USA
SO PCT Int. Appl., 79 pp.
CODEN: PIXXD2
PI WO 9417086 A1 19940804
DS W: CA, JP, US
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
AI WO 94-US348 19940110

PRAI US 93-8897 19930125
DT Patent
LA English
AB Oligonucleotides or oligonucleotides derivs. for use in decreasing the level of transcription of a target gene are described. The target sequence has a substantially sym. purine-rich region that does not necessarily take up a B-conformation and is involved in transcription of the target gene. These oligonucleotides are substantially complementary to the purine stand and do not necessarily form a substantially stable intermol. ***triple*** ***helix*** with the target gene in vitro at physiol. pH. These oligonucleotides may have a circular or stem-loop structure that may form both Watson-Crick and Hoogsteen bonds with the target DNA. These oligonucleotides may be used to control transcription of a gene in the treatment of disease. The method is demonstrated using oligonucleotides targetted against the upstream nuclease-sensitive element of the c-myc gene to inhibit growth of HL60 and K562 cells.

L5 ANSWER 10 OF 32 CA COPYRIGHT 1999 ACS
AN 121:245207 CA
TI ***Triplex*** -mediated inhibition of ***HIV*** DNA integration in vitro
AU Mouscadet, Jean-Francois; Carteau, Sandrine; Goulaouic, Helene; Subra, Frederic; Auclair, Christian
CS Laboratoire de Physicochimie et de Pharmacologie des Macromolecules Biol., Institut Gustave-Roussy, Villejuif, 94805, Fr.
SO J. Biol. Chem. (1994), 269(34), 21635-8
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
AB Integration of human immunodeficiency virus (***HIV***) DNA into the genome of host cells is an obligatory step in the replicative cycle of the virus. The overall process is carried out in vitro by a single viral protein, the integrase, which binds to short sequences located at the ends of viral DNA long terminal repeats (LTRs). These end sequences are highly conserved in all ***HIV*** genomes and are therefore attractive targets for selective DNA binding compds. The integrase-binding site located in U3 LTR contains a purine motif, 5'-GGAAGGG-3' which can be selectively targeted by oligonucleotide-intercalator conjugates. Under neutral pH and physiol. temp., these conjugates readily form a stable complex with the viral DNA which involves a short DNA ***triplex*** . ***Triple*** - ***helix*** formation prevents the catalytic functions of the integrase in vitro which results in a sequence-specific inhibition of the U3 integration process.

L5 ANSWER 11 OF 32 CA COPYRIGHT 1999 ACS

AN 121:197474 CA
TI Effect of abasic linker substitution on ***triplex*** formation, Sp1 binding, and specificity in an oligonucleotide targeted to the human Ha-ras promoter
AU Mayfield, Charles; Miller, Donald
CS Dep. Biochem., Univ. Alabama, Birmingham, AL, USA
SO Nucleic Acids Res. (1994), 22(10), 1909-16
CODEN: NARHAD; ISSN: 0305-1048
DT Journal
LA English
AB A region of the human Ha-ras promoter (-8 to -28) which contains two of the three Sp1 binding sites essential for transcriptional activity forms a sequence specific oligonucleotide-directed pur*pur:pyr ***triple*** ***helix*** . The relative binding of oligonucleotides contg. different substitutions, including an abasic propanediol linker, over three potentially destabilizing C:G interruptions in the otherwise poly G:poly C target was examd. DNase I footprint titrns. reveal that substitution of the pos. charged abasic propanediol linker results in approx. ten fold greater binding than cytosine substitution which in turn provides greater sequence specific binding than substitution of a guanine in the third strand oligonucleotide over the C:G interruptions. Protein binding assays demonstrate that ***triplex*** formation by the linker substituted oligomer (HR21Xap) is less effective in inhibiting Sp1 binding than the cytosine substituted oligomer (HR21ap) both to the target sequence as well as an upstream sequence. As an indication of the effect of linker substitution and targeting consensus Sp1 sites on ***triplex*** specificity, the relative ability of the Ha-ras promoter targeted oligonucleotides to interact with non-target Sp1 sequences within the Ha-ras promoter as well as in the DHFR promoter and ***HIV*** -1 LTR was also investigated. At concns. which afford complete DNase I protection of the target sequence, HR21ap does not bind to the non-target sequences while HR21Xap interacts weakly only at a distal site in the DHFR promoter. Also, HR21ap as well as HR21Xap are specific in their inhibition of Sp1 binding. These results suggest that the propanediol linker is able to skip over interruptions in a target sequence thereby stabilizing ***triplex*** but, slightly compromises sequence specificity and the ability to inhibit Sp1 binding to the Ha-ras promoter.

L5 ANSWER 12 OF 32 CA COPYRIGHT 1999 ACS
AN 121:26881 CA
TI Anti-viral oligomers that bind polypurine tracts of single-stranded RNA or RNA-DNA hybrids
IN Moelling, Karin
PA Apollon, Inc., USA; Max-Planck-Gesselshaft zur Forederung der Wissensc haften E.V.

SO PCT Int. Appl., 83 pp.
CODEN: PIXXD2
PI WO 9407367 A1 19940414
DS W: CA, JP, US
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
AI WO 93-US9300 19930929
PRAI US 92-954184 19920929
DT Patent
LA English
AB The present invention provides methods of inhibiting a virus with which a vertebrate is infected and which replicates via an RNA template comprising the administration of an antisense or ***triplex*** -forming oligonucleotide or a deriv. thereof capable of binding to a polypurine-rich tract (PPT) in a region of single-stranded RNA or RNA-DNA-hybrid, resp. Chimeric oligonucleotides comprising binding domains (A and C) connected by a linker (B), and which are capable of forming ***triplex*** structures with single-stranded nucleic acids are also disclosed. Various PPT-binding antisense nucleic acids were tested for their ability to inhibit cDNA formation from viral RNA, and PPT-binding oligonucleotides of the form A-B-C for their ability to form triplexes with and inhibit RNase H cleavage of viral RNA. Several of the oligonucleotides conferred 100% protection on the single-stranded RNA. One of the oligonucleotides did not exhibit ***triplex*** formation but inhibited RNA degrdn. 100%. The oligonucleotides were added in nanomolar to micromolar amts.

L5 ANSWER 13 OF 32 CA COPYRIGHT 1999 ACS
AN 120:292249 CA
TI ***Triple*** ***Helix*** Formation with Short Oligonucleotide-Intercalator Conjugates Matching the ***HIV*** -1 U3 LTR End Sequence
AU Mouscadet, Jean-Francois; Ketterle, Christophe; Goulaouic, Helene; Carteau, Sandrine; Subra, Frederic; Le Bret, Marc; Auclair, Christian
CS Laboratoire de Physicochimie et de Pharmacologie des Macromolecules Biologiques, Institut Gustave-Roussy, Villejuif, 94805, Fr.
SO Biochemistry (1994), 33(14), 4187-96
CODEN: BICHAW; ISSN: 0006-2960
DT Journal
LA English
OS CJACS
AB In an attempt to target short purine sequences in view of pharmacol. application, the authors have synthesized three new TFO (***triple*** - ***helix*** -forming oligonucleotide) conjugates in which an intercalating oxazolopyridocarbazole (OPC) chromophore is linked by a pentamethylene linker to a 7-mer oligonucleotide matching the polypurine/polypyrimidine sequence located in the ***HIV*** -1 U3 LTR

end region. The TFO moiety of conjugates are 5'CCTTCCC, 5'GGGAAGG, and 5'GGGTTGG. Their ability to bind to double-stranded DNA targets was examd. This binding is demonstrated by a footprinting technique using DNase I as a cleaving agent. The complex involved intermol. pyr-pur*pyr or pur-pur*pyr ***triple*** ***helix*** . Pyrimidine TFO-OPC binds in a pH-dependent manner, whereas the others do not. The formation of the complex has been investigated at neutral pH and increasing temp. The protection due to the purine and mixed TFO-OPC was pH independent and remained identical up to 40 .degree.C. To det. the position of the OPC chromophore, mol. modeling was undertaken on the purine-conjugate/target complex. It has been suggested that the complex involved the intercalation of the OPC at the ***triplex*** -duplex junction with a small unwinding at the next excluded site.

L5 ANSWER 14 OF 32 CA COPYRIGHT 1999 ACS
AN 120:291459 CA
TI Cholesteryl-modified ***triple*** - ***helix*** forming oligonucleotides and their uses
IN Jayaraman, Krishna; Vu, Huynh; Zendegui, Joseph; Hogan, Michael E.
PA Triplex Pharmaceutical Corp., USA; Baylor College of Medicine
SO PCT Int. Appl., 85 pp.
CODEN: PIXXD2
PI WO 9404550 A1 19940303
DS W: CA, JP
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
AI WO 93-US7743 19930817
PRAI US 92-934065 19920821
US 93-53040 19930423
DT Patent
LA English
AB Cholesterol-modified ***triple*** ***helix*** -forming oligonucleotides that have improved ***triple*** ***helix*** -forming properties are described for use in the treatment of disease. The use of a lipophilic moiety improves the passage of the oligonucleotide into cells. 2-Cyanoethyl solketal was synthesized from solketal and acrylonitrile by redn. with NaH in THF and then reduced to 3-aminopropyl solketal with NaBH4 in the presence of Co(II)Cl2. The aminopropyl solketal was then conjugated with cholesteryl chloroformate in pyridine to give N-((cholesteryloxy)carbonyl)-3-aminopropyl solketal that was converted to 1-O(4,4'-dimethoxytrityl)-3-O-(N-(cholesteryloxy) carbonyl-3-aminopropyl) glycerol by reaction with 4,4'-dimethoxytrityl chloride and this was immobilized on controlled-pore glass. Coupling efficiency on the cholesteryl glass was very low; coupling of the 1-O(4,4'-dimethoxytrityl)-3-O-(N-(cholesteryloxy) carbonyl-3-aminopropyl) glycerol to TentaGel-NH2 with with O-benzotriazole-1-yl-N,N,N',N'-

tetramethyluronium tetrafluoroborate, 1-hydroxybenzotriazole hydrate, and N-Et morpholine gave a greatly increased efficiency of coupling. In uptake expts. with animal cell cultures it was found that intracellular concns. of of cholesteryl oligonucleotides exceeded those of the medium. Whole cell uptake of cholesteryl oligonucleotide was 2-22-fold higher than for a control oligonucleotide and nuclear uptake was 3-26-fold higher (depending upon cell type tested.). The cholesteryl oligonucleotides were also more effective at inhibiting expression of the target gene.

L5 ANSWER 15 OF 32 CA COPYRIGHT 1999 ACS
AN 120:237513 CA
TI Identification and characterization of a human herpesvirus 6 gene segment capable of transactivating the human immunodeficiency virus type 1 long terminal repeat in an Sp1 binding site-dependent manner
AU Wang, Jinhai; Jones, Clinton; Norcross, Michael; Bohnlein, Ernst; Razzaque, Abdur
CS Div. Viral Prod., Cent. Biol. Eval. Res., Bethesda, MD, 20892, USA
SO J. Virol. (1994), 68(3), 1706-13
CODEN: JOVIAM; ISSN: 0022-538X
DT Journal
LA English
AB The human immunodeficiency virus type 1 (***HIV*** -1) long terminal repeat (LTR) is transactivated by various extracellular signals and viral cofactors that include human herpesviruses. These transactivators are capable of transactivating the ***HIV*** -1 LTR through the transactivation response element, NF-.kappa.B, or other regulatory binding elements. Human herpesvirus 6 (HHV-6) is a potential cofactor of ***HIV*** -1. An HHV-6 gene segment, ZVH14, which can neoplastically transform NIH 3T3 and human keratinocytes, is capable of transactivating ***HIV*** -1 LTR chloramphenicol acetyltransferase constructs in an Sp1 binding site-dependent manner. Transactivation increased synergistically in the presence of multiple Sp1 sites and was dramatically reduced by cotransfection with oligomers designed to form ***triplex*** structures with ***HIV*** -1 LTR Sp1 binding sites. ***HIV*** -1 LTR NF-.kappa.B sites were not essential for ZVH14-mediated transactivation. A putative open reading frame in ZVH14, B115, which may encode a highly basic peptide consisting of 115 amino acid residues, showed transactivation capacity similar to that of ZVH14. This open reading frame also transactivated the ***HIV*** -1 LTR in an Sp1 site-dependent fashion in African green monkey kidney cells and human T cells. These data suggest that HHV-6 may stimulate ***HIV*** -1 replication via transactivation of Sp1 binding sites present in the ***HIV*** -1 promoter.

L5 ANSWER 16 OF 32 CA COPYRIGHT 1999 ACS

AN 120:208270 CA
TI Anticoagulant activity of sulfated schizophyllan
AU Hirata, Akio; Itoh, Wataru; Tabata, Kengo; Kojima, Tekemasa; Itoyama, Shinji; Sugawara, Isamu
CS Fes. Lab., Taito Co., Ltd., Kobe, 653, Japan
SO Biosci., Biotechnol., Biochem. (1994), 58(2), 406-7
CODEN: BBBIEJ; ISSN: 0916-8451
DT Journal
LA English
AB Sulfated schizophyllans with lower anticoagulant activity and higher anti-***HIV*** activity were prepd. Those with sulfur contents above 6% showed anticoagulant activity irresp. of mol. wt. The activity was correlated with sulfur content. The ***triple*** ***helical*** structure of sulfated schizophyllans did not affect their anticoagulant activity. A sulfated schizophyllan with a sulfur content of 5.0% showed low anticoagulant activity and good anti- ***HIV*** activity. These results indicate that the latter sulfated schizophyllan (sulfur content, 5%) would be useful as an anti- ***HIV*** agent for treatment of ***HIV*** -infected hemophiliacs.

L5 ANSWER 17 OF 32 CA COPYRIGHT 1999 ACS
AN 120:131451 CA
TI Gene therapy for AIDS
AU Nagayama, Hitomi; Tani, Kenzaburo
CS Inst. Med. Sci., Univ. Tokyo, Tokyo, 108, Japan
SO Mol. Med. (Tokyo) (1993), 30(12), 1558-60
CODEN: MOLMEL; ISSN: 0918-6557
DT Journal; General Review
LA Japanese
AB A review, with 8 refs., on the results and problems to be solved in gene therapy of ***HIV*** infection; antisense method, RNA decoy using TAR (tat binding motif), ribozyme cutting gag RNA or 5' leader sequence, mutant gene methods using dominant neg. effects on rev gene, and ***triple*** - ***helix*** formation method. Gene therapy using env gene is involved in cellular immunity.

L5 ANSWER 18 OF 32 CA COPYRIGHT 1999 ACS
AN 120:23569 CA
TI ***Triple*** ***helix*** recognition of DNA
IN Dervan, Peter B.
PA California Institute of Technology, USA
SO PCT Int. Appl., 42 pp.
CODEN: PIXXD2
PI WO 9318187 A1 19930916
DS W: CA, JP

RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
AI WO 93-US2352 19930311
PRAI US 92-850503 19920313
DT Patent
LA English
AB Described are oligonucleotides and processes for their use for specific recognition of a target sequence in double-stranded nucleic acid through the formation of a ***triple*** ***helix*** . The oligonucleotides contain nebularine (N) and bind to one of the strands of the target sequence. The oligonucleotides can be used as diagnostic or therapeutic agents through incorporation of an appropriate group in .gtoreq.1 nucleotides in the ***triple*** ***helix*** -forming oligonucleotide. The purine-rich target sequence 5'-d(AGAG2CGAG4CG2)-3'.bul.5'-d(C2GC4TCGC2TCT)-3', a sequence occurring in the ***HIV*** genome, was prepd. with plasmid pULHIV by recombinant methods. A restriction fragment contg. the target sequence was reacted with 5'-d(G2Z2G4Z1GZ2G2Z1GT*)-3' [Z1 = A, T, N; Z2 = N, A, G, C, T; T* = thymidine-EDTA.bul.Fe(II)] (I) in the presence of dithiothreitol at 37.degree.. One major cleavage product indicated sequence-specific cleavage was only obsd. for 4 of I.

L5 ANSWER 19 OF 32 CA COPYRIGHT 1999 ACS
AN 120:2227 CA
TI Oligonucleotide clamps arrest DNA synthesis on a single-stranded DNA target
AU Giovannangeli, Carine; Thuong, Nguyen T.; Helene, Claude
CS Lab. Biophys., Cent. Natl. Rech. Sci., Paris, 75231, Fr.
SO Proc. Natl. Acad. Sci. U. S. A. (1993), 90(21), 10013-17
CODEN: PNASA6; ISSN: 0027-8424
DT Journal
LA English
AB ***Triple*** ***helixes*** can be formed on single-stranded oligopurine target sequences by composite oligonucleotides consisting of two oligonucleotides covalently linked by either a hexaethylene glycol linker or an oligonucleotide sequence. The first oligomer forms Watson-Crick base pairs with the target, while the second oligomer engages in Hoogsteen base pairing, thereby acting as a mol. clamp. The ***triple*** - ***helical*** complex formed by such an oligonucleotide clamp, or "oligonucleotide-loop-oligonucleotide" (OLO), is more stable than either the corresponding trimol. ***triple*** ***helix*** or the double helix formed upon binding of the oligopyrimidine complement to the same oligopurine target. Attaching a psoralen deriv. to the 5' end of the OLO allowed to photoinduce a covalent linkage to the target sequence. The psoralen moiety became covalently linked to all three portions of the ***triplex*** , thereby making the oligonucleotide clamp irreversible.

These crosslinking reactions introduced strong stop signals during DNA replication, as shown on a plasmid contg. a portion of the ***HIV*** proviral sequence of human immunodeficiency virus. A 16-mer oligopurine sequence corresponding to the polypurine tract of human immunodeficiency virus was chosen as a target for a psoralen-OLO conjugate. Three different stop signals for DNA polymerase were obsd., corresponding to different sites of polymerase arrest on its template. Even in the absence of photoinduced crosslinking, the psoralen-OLO conjugate was able to arrest DNA replication. The formation of ***triple*** - ***helical*** structures on single-stranded targets may provide an alternative to the antisense strategy for the control of gene expression.

L5 ANSWER 20 OF 32 CA COPYRIGHT 1999 ACS
AN 119:203763 CA
TI Purine base modified 2'-deoxyribonucleosides, use in ***triplex*** -forming oligonucleotides and process for preparing the same
IN Revankar, Ganapathi Ramakrishna; Hogan, Michael Edward; Rao, Takkellapati Sudhakar; Shroff, Hitesh Navinchandra
PA Triplex Pharmaceutical Corp., USA; Baylor College of Medicine
SO PCT Int. Appl., 76 pp.
CODEN: PIXXD2
PI WO 9221690 A1 19921210
DS W: CA, JP, NO
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE
AI WO 92-US4795 19920604
PRAI US 91-712151 19910605
DT Patent
LA English
OS MARPAT 119:203763
AB Title compds., esp. the deoxyformycin deriv. I, were prepd. for incorporation into ***triplex*** -forming oligonucleotides. Thus, I was prepd. from formycin and was incorporated into an ***HIV*** -1 mRNA fragment to give the sequence
5'-TGGGTGGFGTGGFFTGGGFGGGTFTGGGGTGTGGFGTG-3'
(F = deoxyformycin nucleotide). This sequence is useful in treating ***HIV*** -1 infection (no data).

L5 ANSWER 21 OF 32 CA COPYRIGHT 1999 ACS
AN 119:175237 CA
TI Unexpected effect of an anti-human immunodeficiency virus intermolecular ***triplex*** -forming oligonucleotide in an in vitro transcription system due to RNase H-induced cleavage of the RNA transcript
AU Praseuth, Daniele; Guieysse, Anne Laure; Itkes, Alexander Veniamine; Helene, Claude
CS Lab. Biophys., Mus. Hist. Nat., Paris, 75 231, Fr.

SO Antisense Res. Dev. (1993), 3(1), 33-44
CODEN: AREDEI; ISSN: 1050-5261
DT Journal
LA English
AB A 16-mer oligodeoxynucleotide (ODN) which specifically recognizes the polypurine tract (PPT) located upstream of the 3' long terminal repeat (LTR) of human immunodeficiency virus (***HIV***) proviral DNA via ***triplex*** formation is shown to have a dramatic effect on in vitro transcription from the ***HIV*** -LTR promoter. In the presence of HeLa cell exts., a shorter RNA transcript is obtained in the presence of the 16-mer ODN. This truncated RNA lacks about 200 nucleotides from its 3' region. The PPT sequence is not responsible for this effect. Instead, this process involves a purine-rich sequence in the gag mRNA located around position +400. The imperfect hybrid formed between the 16-mer ODN and mRNA is precisely cleaved by RNase H contained in HeLa cell exts. These data show that sophisticated control expts. must be designed before any conclusion can be drawn on the effect of oligonucleotides used in vitro and in cell cultures.

L5 ANSWER 22 OF 32 CA COPYRIGHT 1999 ACS
AN 119:173630 CA
TI The polypurine tract, PPT, of ***HIV*** as target for antisense and ***triple*** - ***helix*** -forming oligonucleotides
AU Volkmann, S.; Dannull, J.; Moelling, K.
CS Abt. Schuster, Max-Planck-Inst. Mol. Genet., Berlin, D-1000/33, Germany
SO Biochimie (1993), 75(1-2), 71-8
CODEN: BICMBE; ISSN: 0300-9084
DT Journal
LA English
AB Replication of retroviral DNA into double-stranded DNA provirus involves initiation of plus-strand DNA synthesis at the polypurine tract, PPT, by the reverse transcriptase (RT). The PPT is highly conserved among the known ***HIV*** -1 retroviral isolates. It occurs twice, once within the coding region of the integrase and the other one adjacent to the 3' LTR. The data presented show that two antisense oligonucleotides, a 20-mer and a 40-mer, complementary to the PPT induce complete blocks of DNA synthesis whereas an antisense oligonucleotide outside the PPT is only slightly inhibitory. Previously, polypurine sequences have been used by several groups for ***triplex*** -formation. During replication, the ***HIV*** -polypurine tract, PPT, is present in a RNA-DNA hybrid. Therefore ***triple*** - ***helix*** formation consisting of RNA-DNA and a third DNA strand covering the PPT region was tested here by protection against RNase H cleavage in vitro. Incubation with a pyrimidine oligonucleotide in parallel orientation to the PPT-RNA shows some protection. GT-pyrimidine-purine mixed oligonucleotides (25-mer) led

to protection against RNase H up to 50% independent of their orientation. The data suggest that ***triple*** - ***helix*** formation may have taken place with the PPT in vitro. Therefore, this highly conserved structure may prove useful in nucleic acid based anti-viral therapy with antisense or ***triple*** - ***helix*** approaches. Furthermore, the influence of ***HIV*** -1 nucleocapsid (NC) protein, NCp15, on reverse transcription is reported. The data show a two- to three-fold stimulatory effect of the NCp15 on RNA directed DNA synthesis.

L5 ANSWER 23 OF 32 CA COPYRIGHT 1999 ACS
AN 119:173623 CA
TI ***Triple*** - ***helix*** formation by oligonucleotides containing the three bases thymine, cytosine, and guanine
AU Giovannangeli, Carine; Rougee, Michel; Garestier, Therese; Thuong, Nguyen T.; Helene, Claude
CS Cent. Natl. Rech. Sci., Inst. Natl. Sante Rech., Paris, 75231, Fr.
SO Proc. Natl. Acad. Sci. U. S. A. (1992), 89(18), 8631-5
CODEN: PNASA6; ISSN: 0027-8424
DT Journal
LA English
AB A homopurine-homopyrimidine sequence of human immunodeficiency virus (***HIV***) proviral DNA was chosen as a target for ***triple*** - ***helix*** -forming oligonucleotides. An oligonucleotide contg. three bases (thymine, cytosine, and guanine) was shown to bind to its target sequence under physiol. conditions. This oligonucleotide is bound in a parallel orientation with respect to the homopurine sequence. Thymines recognize A.T base pairs to form T.A.T base triplets and guanines recognize a run of G.C base pairs to form G.G.C base triplets. A single 5-methylcytosine was shown to stabilize the ***triple*** ***helix*** when incorporated in a stretch of thymines; it recognizes a single G.C base pair in a run of A.T base pairs. These results provide some of the rules required for choosing the more appropriate oligonucleotide sequence to form a ***triple*** ***helix*** at a homopurine-homopyrimidine sequence of duplex DNA. A psoralen deriv. attached to the oligonucleotide contg. thymine, 5-methylcytosine, and guanine was shown to photoinduce crosslinking of the two DNA strands at the target sequence in a plasmid contg. part of the ***HIV*** proviral DNA sequence. ***Triplex*** formation and crosslinking were monitored by inhibition of Dra I restriction enzyme cleavage. The present results provide a rational basis for the development of ***triplex*** -forming oligonucleotides targeted to specific sequences of the ***HIV*** provirus integrated in its host genome.

L5 ANSWER 24 OF 32 CA COPYRIGHT 1999 ACS
AN 119:154917 CA

TI Oligodeoxynucleotide-directed photo-induced cross-linking of ***HIV*** proviral DNA via ***triple*** - ***helix*** formation
AU Giovannangeli, Carine; Thuong, Nguyen T.; Helene, Claude
CS Lab. Biophys., Mus. Natl. Histoire Nat., Paris, 75231, Fr.
SO Nucleic Acids Res. (1992), 20(16), 4275-81
CODEN: NARHAD; ISSN: 0305-1048
DT Journal
LA English
AB The ***HIV*** proviral genome contains two copies of a 16 bp homopurine.cntdot.homopyrimidine sequence which overlaps the recognition and cleavage site of the Dra I restriction enzyme. Psoralen was attached to the 16-mer homopyrimidine oligonucleotide, d5'(TTTTCT-TTTCCCCCCT)3', which forms a ***triple*** ***helix*** with this ***HIV*** proviral sequence. Two plasmids, contg. part of the ***HIV*** proviral DNA, with either one (pLTR) or two (pBT1) copies of the 16-bp homopurine.cntdot.homopyrimidine sequence and either 4 or 14 Dra I cleavage sites, resp., were used as substrates for the psoralen-oligonucleotide conjugate. Following UV irradn. the two strands of the DNA targeted sequence were crosslinked at the ***triplex*** -duplex junction. The psoralen-oligonucleotide conjugate selectively inhibited Dra I enzymic cleavage at sites overlapping the two ***triple*** ***helix*** -forming sequences. A secondary ***triplex*** -forming site of 8 contiguous base pairs was obsd. on the pBT1 plasmid when binding of the 16 base-long oligonucleotide was allowed to take place at high oligonucleotide concns. Replacement of a stretch of six cytosines in the 16-mer oligomer by a stretch of six guanines increased binding to the primary sites and abolished binding to the secondary site under physiol. conditions. These results demonstrate that oligonucleotides can be designed to selectively recognize and modify specific sequences in ***HIV*** proviral DNA.

L5 ANSWER 25 OF 32 CA COPYRIGHT 1999 ACS
AN 119:133885 CA
TI ***Triple*** - ***helix*** formation at purine-pyrimidine tracts and its use in nucleic acid cleavage
IN Jayasena, Sumedha D.; Johnston, Brian H.
PA SRI International, USA
SO PCT Int. Appl., 101 pp.
CODEN: PIXXD2
PI WO 9312230 A1 19930624
DS W: CA, JP
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
AI WO 92-US10792 19921211
PRAI US 91-808452 19911213
US 92-826934 19920121

DT Patent
LA English
AB An oligonucleotide with an alternating pyrimidine-purine tract is able to form a ***triple*** - ***helix*** with a duplex nucleic acid mol. contg. a complementary purine-pyrimidine tract. Both tracts contain .gtoreq.4 pyrimidines and purines, resp. The oligonucleotides can be conjugated with a cleaving agent, e.g., phenanthroline Cu(II), and used for cleaving a target duplex nucleic acid. A single-stranded nucleic acid mol. may also be cleaved by using an oligonucleotide contg. 3 regions capable of forming ***triplex*** -helix. In vitro cleavage of the LTR sequence of ***HIV*** -1 with a phenanthroline-coupled oligonucleotide, in which the cleavage was initiated with CuSO4 and mercaptopropionic acid, was demonstrated. The method can be used for diagnosis of viral infection and controlling of viral gene expression.

L5 ANSWER 26 OF 32 CA COPYRIGHT 1999 ACS
AN 119:133655 CA
TI Cation and sequence effects on stability of intermolecular pyrimidine-purine-purine ***triplex***
AU Malkov, V. A.; Voloshin, O. N.; Soyfer, Valery N.; Frank-Kamenetskii, D.
CS Inst. Mol. Genet., Moscow, 123182, Russia
SO Nucleic Acids Res. (1993), 21(3), 585-91
CODEN: NARHAD; ISSN: 0305-1048
DT Journal
LA English
AB A differential effect is found of various bivalent cations (Ba2+, Ca2+, Mg2+, Cd2+, Co2+, Mn2+, Ni2+, Zn2+ and Hg2+) on the stability of intermol. Py-Pu-Pu triplexes with different sequences of base triads. Ca2+, Mg2+, Cd2+, Co2+, Mn2+, Ni2+ and Zn2+ stabilize the d(C)n d(G)n d(G)n ***triplex*** whereas Ba2+ and Hg2+ do not. Ba2+, Ca2+, Mg2+ and Hg2+ destabilize the d(TC)n d(GA)n d(AG)n ***triplex*** whereas Cd2+, Co2+, Mn2+, Ni2+ and Zn2+ stabilize it. The complexes obsd. are rather stable because they do not dissoc. during gel electrophoresis in co-migration expts. Chem. probing expts. with di-Me sulfate as a probe indicate that an arbitrary homopurine-homopyrimidine sequence forms a ***triplex*** with the corresponding purine oligonucleotide in the presence of Mn2+ or Zn2+, but not Mg2+. In the complex the purine oligonucleotide has antiparallel orientation with respect to the purine strand of the duplex. Specifically, the Py-Pu-Pu ***triplex*** formed in a fragment of human papilloma virus ***HPV*** -16 in the presence of Mn2+.

L5 ANSWER 27 OF 32 CA COPYRIGHT 1999 ACS
AN 118:227520 CA
TI Nucleic acid sequence detection by ***triple*** ***helix*** formation

IN Vary, Calvin P. H.
PA Idexx Laboratories, Inc., USA
SO PCT Int. Appl., 80 pp.
CODEN: PIXXD2
PI WO 9211390 A1 19920709
DS W: CA, JP
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE
AI WO 91-US9402 19911211
PRAI US 90-629601 19901217
DT Patent
LA English
AB A method is disclosed for detection of a nucleic acid target sequence by formation of ***triple*** ***helix*** nucleic acid structures. The method may, but need not, involve amplification of the nucleic acid in vitro using cycles of denaturation and amplification (esp. by PCR or ligase chain reaction) to yield product duplexes, and detecting the product duplexes by hybridizing a third strand of nucleic acid to the product duplexes without denaturation. The ***triple*** ***helix*** -forming sequences may be endogenous to the target sequence being detected, or they may be introduced in the probes used during amplification. The method of the invention was used to detect M. paratuberculosis, caprine arthritis encephalitis virus, and human immunodeficiency virus 1.

L5 ANSWER 28 OF 32 CA COPYRIGHT 1999 ACS
AN 117:85420 CA
TI Design of novel bases on recognition of GC base pairs of DNA by oligonucleotide directed ***triple*** ***helix*** formation
AU Koh, J. S.; Dervan, P. B.
CS Div. Chem. Chem. Eng., California Inst. Technol., Pasadena, CA, USA
SO Report (1991), Order No. AD-A237529, 16 pp. Avail.: NTIS
From: Gov. Rep. Announce. Index (U. S.) 1991, 91(21), Abstr. No. 159,424
DT Report
LA English
AB The binding of pyrimidine oligonucleotides contg. cytosine at single sites on double helical DNA by ***triple*** ***helix*** formation is sensitive to pH. An important factor is the required protonation of the cytosine N-3 in the third strand to enable the formation of two Hoogsteen hydrogen bonds (G + GC triplet). Because oligonucleotide specificity could provide a method for artificial repression of viral and pathogenic diseases, it is desirable to design and synthesize a novel base that could bind GC base pairs strongly and selectively over a wide range of intracellular pH. The novel base, N-1-15-O-(bis(4-methoxyphenyl)phenylmethyl-2-deoxy-beta-D-erythro-pentofuranosyl-4,7-dihydro-3-methyl-7-oxo-1H-pyrazolo-4,3-dipyrimidine-5-yl-2-

methylpropanamide (P1) was designed, synthesized and incorporated within a pyrimidine oligonucleotide and shown to recognize GC base pairs as selectively and strongly as C. Oligonucleotides contg. P1 bases show the same specificity as C but with less pH sensitivity in ***triple*** ***helix*** formation. P1 does not require protonation in ***triple*** ***helix*** formation. Such specificity allows binding at a 15 base pairs site in plasmid DNA (pH 7.8) and a 16 base pairs site in the 3' long terminal repeat (LTR) of ***HIV*** DNA (pH 7.4).

L5 ANSWER 29 OF 32 CA COPYRIGHT 1999 ACS
AN 117:27030 CA
TI Synthesis and base-pairing properties of the nuclease-resistant .alpha.-anomeric dodecaribonucleotide .alpha.-[r(UCUUAACCCACA)]
AU Debart, Francoise; Rayner, Bernard; Degols, Genevieve; Imbach, Jean Louis
CS Lab. Chim. Bio-Org., Univ. Montpellier II, Montpellier, 34095, Fr.
SO Nucleic Acids Res. (1992), 20(6), 1193-200
CODEN: NARHAD; ISSN: 0305-1048
DT Journal
LA English
AB The non natural oligoribonucleotide .alpha.-[r(UCUUAACCCACA)] consisting exclusively of .alpha.-anomeric ribonucleoside units was synthesized according to the phosphoramidite methodol. and the solid support technol. For this purpose, the base-protected .alpha.-ribonucleosides were synthesized and converted into their O-methylphosphoramidites. Assembling was carried out on a DNA synthesizer with an av. efficiency of 97% per step. Base compn. of this nuclease-resistant .alpha.-RNA strand was ascertained after chem. and enzymic hydrolysis and HPLC anal. of the hydrolyzate. Whereas no spectroscopic evidence of base pairing was found above 0.degree. between .alpha.-[r(UCUUAACCCACA)] and .beta.-[d(TGTGGGTTAAGA)], a clear UV absorbance transition was obsd. during the hybridization of the same .alpha.-RNA strand with .beta.-[d(AGAATTGGGTGT)]. In this latter case, the mixing curve titrn. suggests formation at low temp. of a ***triplex*** involving two .alpha.-RNA and one .beta.-NA strands. Moreover, this .alpha.-decaribonucleotide complementary in parallel orientation of the splice receptor of ***HIV*** -1 mRNA was found to inhibit with apparent lack of sequence specificity, in de novo ***HIV*** -1 infection in cultured cells.

L5 ANSWER 30 OF 32 CA COPYRIGHT 1999 ACS
AN 116:247935 CA
TI Inhibition of transcription of ***HIV*** -1 in infected human cells by oligodeoxynucleotides designed to form DNA ***triple*** ***helixes***
AU McShan, W. Michael; Rossen, Roger D.; Laughter, Arline H.; Trial, JoAnn; Kessler, Donald J.; Zendegui, Joseph G.; Hogan, Michael E.; Orson, Frank

M.
CS Veterans Affairs Med. Cent., Houston, TX, 77030, USA
SO J. Biol. Chem. (1992), 267(8), 5712-21
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
AB The effect on human immunodeficiency virus 1 (***HIV*** -1) viral transcription and subsequent gene expression mediated by mixed purine-pyrimidine oligodeoxyribonucleotides (oligodeoxynucleotides) designed to form collinear DNA triplexes with purine-rich elements in the viral promoter was evaluated in intact mammalian cell lines (MT4 and U937). Oligonucleotides HIV31 (5'-GTTTTTGGGTGTTGTGGGTGTGTGTGGTTTG-3') and HIV38 (5'-TGGGTGGGGTGGGGTGGGGGGGTGTGGGGTGTGGGGTG-3') were designed to interact with the transcription initiation site (-16 to +13) and nuclear factor Sp1 binding site (-81 to -44) of ***HIV*** -1, resp. Oligonucleotides, synthesized with a 3' amine blocking group (5-R-O-PO2-OCH(CH2OH)-CH2-NH3+-3') to prevent degrdn. by cellular nucleases, were readily taken up by MT4 cells from the culture medium, achieving measured intranuclear concns. higher than the medium in less than 2 h of incubation. The 3' amine-modified oligonucleotides were recoverable from the cells after 24 h as >90% intact material. Treatment of acutely infected MT4 cells with either HIV31 or HIV38 significantly inhibited viral-assocd. cytopathol. and P24 antigen prodn. Addnl., inhibition of P24 antigen release, culture supernatant viral titer, and expression of the intact 9.2-kb ***HIV*** -1 mRNA was obsd. when the chronically infected promonocyte cell line, U937, was treated with 10 .mu.M HIV38. Control oligonucleotides with similar base compn. did not inhibit virus expression in either cell line. Furthermore, inhibition of viral expression was not due to .alpha.-interferon induction resulting from oligonucleotide treatment. Both HIV31 and HIV38 assoc. with their resp. DNA target duplexes at micromolar concns., and a strong neg. ellipticity near 210 nm, characteristic of DNA triplexes, was obsd. in the CD spectrum of either target-oligonucleotide complex. These observations suggest that oligonucleotides, designed to form nucleic acid triplexes with specific proviral target sequences, can selectively inhibit transcription of viral mRNA in intact cells and suppress accumulation of viral products.

L5 ANSWER 31 OF 32 CA COPYRIGHT 1999 ACS
AN 115:201334 CA
TI ***Triple*** - ***helix*** formation by .alpha. oligodeoxynucleotides and .alpha. oligodeoxynucleotide-intercalator conjugates

AU Sun, J. S.; Giovannangeli, C.; Francois, J. C.; Kurfurst, R.; Montenay-Garestier, T.; Asseline, U.; Saison-Behmoaras, T.; Thuong, N. T.; Helene, C.
CS Lab. Biophys., Mus. Natl. Histoire Nat., Paris, 75005, Fr.
SO Proc. Natl. Acad. Sci. U. S. A. (1991), 88(14), 6023-7
CODEN: PNASA6; ISSN: 0027-8424
DT Journal
LA English
AB Base-pair sequences in double-stranded DNA can be recognized by homopyrimidine oligonucleotides that bind to the major groove at homopurine.cntdot.homopyrimidine sequences thereby forming a local ***triple*** ***helix*** . To make oligodeoxynucleotides resistant to nucleases, the natural (.beta.) anomers of the nucleotide units were replaced by the synthetic (.alpha.) anomers. The 11-mer .alpha. oligodeoxynucleotide 5'-d(TCTCCTCCTTT)-3' binds to the major groove of DNA in an antiparallel orientation with respect to the homopurine strand, whereas a .beta. oligonucleotide adopts a parallel orientation. When an intercalating agent was attached to the 3' end of the .alpha. oligodeoxynucleotide, a strong stabilization of the ***triple*** ***helix*** was obsd. A 16-base-pair homopurine.cntdot.homopyrimidine sequence of human immunodeficiency virus proviral DNA was chosen as a target for a 16-mer homopyrimidine .alpha. oligodeoxynucleotide. A restriction enzyme that cleaves DNA at the junction of the homopurine.cntdot.homopyrimidine sequence was inhibited by ***triple*** - ***helix*** formation. The 16-mer .alpha. oligodeoxynucleotide substituted by an intercalating agent was .apprxeq.20 times more efficient than the unsubstituted oligomer. Nuclease-resistant .alpha. oligodeoxynucleotides offer addnl. possibilities to control gene expression at the DNA level.

L5 ANSWER 32 OF 32 CA COPYRIGHT 1999 ACS
AN 115:4696 CA
TI Method for making synthetic oligonucleotides which bind specifically to target sites on duplex DNA molecules, by forming a colinear ***triplex*** , the synthetic oligonucleotides and methods of use
IN Hogan, Michael Edward; Kessler, Donald Joseph
PA Baylor College of Medicine, USA
SO Eur. Pat. Appl., 40 pp.
CODEN: EPXXDW
PI EP 375408 A1 19900627
DS R: GR
AI EP 89-313391 19891220
PRAI US 88-287359 19881220
DT Patent
LA English

AB A method for making synthetic oligonucleotides which bind to target sequences in a duplex DNA forming colinear triplexes by binding to the major groove is disclosed. The method includes scanning genomic duplex DNA and identifying nucleotide target sequences .gtoreq.20 nucleotides having either .gtoreq.65% purine bases or .gtoreq.65% pyrimidine bases; and synthesizing synthetic oligonucleotides complementary to identified target sequences. The synthetic oligonucleotides have a G when the complementary location in the DNA duplex has a GC base pair and have a T when the complementary location in the DNA duplex has an AT base pair. The synthetic oligonucleotides are oriented 5' to 3' and bind parallel or 3' to 5' and bind antiparallel to the .gtoreq.65% purine strand. Also described are synthetic oligonucleotides made by the above methods. The oligonucleotides can be altered by modifying and/or changing the bases, adding linkers and modifying groups to the 5' and/or 3' termini, and changing the backbone. These synthetic oligonucleotides bind to duplex DNA to form triplexes. This process alters the functioning of the genes which are bound, and can be used to inhibit cell growth, alter protein ratios, treat diseases including cancer, and permanently alter the DNA. Oligonucleotides 3'-GTTTTTGGGTGTTGTGGGTGTGTGTGGTT-5' (HIV29par) and 5'-GTTTTTGGGTGTTGTGGGTGTGTGTGGTTTG-3' (HIV31 anti), designed to bind within the major groove of the DNA helix and form triplexes with specific sequences in the tar region of the human immunodeficiency virus 1 (***HIV*** -1) provirus were readily taken up by ***HIV*** -1 infected cells and selectively suppressed synthesis of ***HIV*** -1 mRNA without concomitant suppression of mRNA for a constituent gene of the cells. Inhibition of viral mRNA was dependent on the dose of oligonucleotide added; max. inhibition occurred at 10 mM.

L9 ANSWER 1 OF 77 CA COPYRIGHT 1999 ACS
AN 125:131402 CA
TI The role of nucleic acid in the resistance of ***HIV*** -1 reverse transcriptase to nucleoside and nonnucleoside inhibitors
AU Hsiou, Yu; Das, Kalyan; Ding, Jianping; Boyer, Paul L.; Hughes, Stephen H.; Arnold, Edward
CS Center Advanced Biotechnology Medicine, Piscataway, NJ, 08854-5638, USA
SO Med., Biol., Environ. (1995), 23(2), 209-215
CODEN: MBENDX; ISSN: 0302-0800
DT Journal; General Review
LA English
AB A review with 23 refs. An increasing body of structural, biochem., and clin. data indicates that ***HIV*** -1 RT resistance to nucleoside and nonnucleoside inhibitors often involves interactions between spatially distant sites on the enzyme that are mediated by template-primer. A no. of different types of three-dimensional structures of ***HIV*** -1 RT have been detd. These include the structures of complexes of ***HIV***

-1 RT with ***double*** - ***stranded*** nucleic acid, with nonnucleoside inhibitors, and without bound ligands. An examn. of these structures have suggested that most of the positions where mutations give rise to nucleoside resistance do not affect dNTP ***binding*** directly. Instead, the nucleoside-resistance mutations may affect the geometry of the dNTP- ***binding*** site indirectly by interacting with nucleic acid. Resistance to nucleotide analogs requires that the enzyme is able to discriminate between normal nucleotide substrates and the analog(s)-we believe that this occurs because the active site for the polymerase is composed of both protein and nucleic acid. In contrast to the nucleoside-resistance mutations, most of the residues involved in resistance to nonnucleoside inhibitors are located in close proximity to the drug- ***binding*** pocket. However, the ***binding*** sites for both classes of inhibitors and the template-primer are proximal, and it is likely that the resistance of ***HIV*** -1 RT to both classes of drugs involves an interplay of protein, nucleic acid, and the inhibitors. Clin. and biochem. studies of the resistance of ***HIV*** -1 RT to nucleoside and nonnucleoside inhibitors will be described in relation to the three-dimensional structure of ***HIV*** -1 RT and its interactions with nucleic acid. These observations have important implications for combination therapy using different classes of ***HIV*** -1 RT inhibitors since the ability of the enzyme to undergo mutation is constrained both by complexity of its functional requirements and by the intersecting roles of the amino acids involved in ***binding*** inhibitors and template-primer.

L9 ANSWER 2 OF 77 CA COPYRIGHT 1999 ACS
AN 124:136977 CA
TI Expression of TAR RNA- ***binding*** protein in baculovirus and co-immunoprecipitation with insect cell protein kinase
AU Blair, Edward D.; Roberts, Christopher M.; Snowden, B. Wendy; Gatignol, Anne; Benkirane, Monsef; Jeang, Kuan-Teh
CS Wellcome Research Laboratories, Beckenham, BR3 3BS, UK
SO J. Biomed. Sci. (Basel) (1995), Volume Date 1995, 2(4), 322-9
CODEN: JBCIEA; ISSN: 1021-7770
DT Journal
LA English
AB TAR RNA- ***binding*** protein TRBP was originally isolated by its ***binding*** affinity for radiolabeled ***HIV*** -1 leader RNA. Subsequent studies have suggested that this protein is one member of a family of ***double*** - ***stranded*** RNA- ***binding*** proteins. Recent findings indicate that TRBP might function to antagonize the translational inhibitory effect that can be mediated through cellular protein kinase, PKR. Here, we report on the over-expression of a cDNA coding for TRBP in eukaryotic SF9 cells using baculovirus. We

characterized the nuclear localization of TRBP in insect cells, and we demonstrate that TRBP co-immunoppts. with a protein in these cells antigenically related to human PKR.

L9 ANSWER 3 OF 77 CA COPYRIGHT 1999 ACS
AN 123:337292 CA
TI ***HIV*** -1 Tat directly interacts with the interferon-induced, ***double*** - ***stranded*** RNA-dependent kinase, PKR
AU McMillan, Nigel A.; Chun, Rene F.; Siderovski, David P.; Galabru, Julien; Toone, W. Mark; Samuel, Charles E.; Mak, Tak W.; Hovanessian, Ara G.; Jeang, Kuan-Teh; Williams, Bryan R. G.
CS Dep. of Cancer Biology, Cleveland Clinic Foundation, Cleveland, OH, 44195, USA
SO Virology (1995), 213(2), 413-24
CODEN: VIRLAX; ISSN: 0042-6822
DT Journal
LA English
AB We present evidence that the ***HIV*** -1 Tat protein and the RNA-dependent cellular protein kinase, PKR, interact with each other both in vitro and in vivo. Using GST fusion chromatog., we demonstrate that PKR, interacts directly with the ***HIV*** -1 Tat protein. The region in Tat sufficient for ***binding*** PKR maps within amino acids 20 to 72. In vitro assays, the two-exon form of Tat (Tat 86) was phosphorylated by PKR, while the one exon form of TAT (Tat 72) inhibited PKR autophosphorylation and substrate phosphorylation. The ability of Tat to interact with PKR was demonstrated in both yeast and mammalian cells. Expression of PKR in yeast results in a growth suppressor phenotype which was reversed by coexpression of a one exon form of Tat. Expression of Tat 82 in HeLa cells resulted in direct interaction with PKR as detected by coimmunopptn. with a Tat antibody. Tat and PKR also form a coimmunoprecipitable complex in cell-free exts. prepd. from productively infected T lymphocytes. The interaction of Tat with PKR provides a potential mechanism by which ***HIV*** could suppress the interferon system.

L9 ANSWER 4 OF 77 CA COPYRIGHT 1999 ACS
AN 123:279476 CA
TI Isolation of high-affinity RNA ligands to ***HIV*** -1 integrase from a random pool
AU Allen, Patrick; Worland, Steve; Gold, Larry
CS Department of Molecular, Cellular and Developmental Biology, University of Colorado at Boulder, Boulder, CO, 80309-0347, USA
SO Virology (1995), 209(2), 327-36
CODEN: VIRLAX; ISSN: 0042-6822
DT Journal

LA English
AB High-affinity RNAs were isolated from a random pool that ***binds*** to integrase protein from the human immunodeficiency virus-type 1 by using the procedure now known as SELEX. Generally, the RNAs fell into 3 different classes in ***binding*** buffer contg. 250 mM NaCl: group I class of mols. ***binds*** integrase with a dissocn. const. (Kd) on the order of 10 nM, group II mols. had a Kd of about 80 nM, and group III about 800 nM. The RNA with the highest affinity from the group I class of mols., designated P5, was characterized using computer modeling, chem. and enzymic probing, and deletion anal. The secondary structure model for this RNA suggests interactions between looped-out fixed nucleotides and nucleotides from the randomized region; a GNRA tetraloop is also in the structure. Integrase was able to process a U5 mimic in vitro. P5 competes effectively for ***binding*** with the ***double*** - ***stranded*** DNA mimic of U5 at 180 mM NaCl concn.

L9 ANSWER 5 OF 77 CA COPYRIGHT 1999 ACS
AN 123:221737 CA
TI Mechanistic implications from the structure of a catalytic fragment of Moloney murine leukemia virus reverse transcriptase
AU Georgiadis, Millie M.; Jessen, Sven M.; Ogata, Craig M.; Telesnitsky, Alice; Goff, Stephen P.; Hendrickson, Wayne A.
CS Waksman Inst., Rutgers Univ., Piscataway, NJ, 08855, USA
SO Structure (London) (1995), 3(9), 879-92
CODEN: STRUE6; ISSN: 0969-2126
DT Journal
LA English
AB Reverse transcriptase (RT) converts the single-stranded RNA genome of a retrovirus into a ***double*** - ***stranded*** DNA copy for integration into the host genome. This process requires RNase H as well as RNA- and DNA-directed DNA polymerase activities. Although the overall organization of ***HIV*** -1 RT is known from previously reported crystal structures, no structure of a complex including a metal ion, which is essential for its catalytic activity, has been reported. Here, the authors describe the crystal structures at 1.8 .ANG. resoln. of a catalytically active fragment of RT from Moloney murine leukemia virus (MMLV) and at 2.6 .ANG. of a complex of this fragment with Mn2+ coordinated in the polymerase active site. On the basis of similarities with ***HIV*** -1 RT and rat DNA polymerase-.beta., the authors modeled template/primer and deoxyribonucleoside 5'-triphosphate substrates into the MMLV RT structure. The model, in the context of the disposition of evolutionarily conserved residues seen here at high resoln., provided new insights into the mechanisms of catalysis, fidelity, processivity, and discrimination between deoxyribose and ribose nucleotides.

L9 ANSWER 6 OF 77 CA COPYRIGHT 1999 ACS
AN 123:220184 CA
TI Cloning and characterization of a novel cellular protein, TDP-43, that ***binds*** to human immunodeficiency virus type 1 TAR DNA sequence motifs
AU Ou, S.-H. Ignatius; Wu, Foon; Harrich, David; Garcia-Martinez, Leon F.; Gaynor, Richard B.
CS Division of Molecular Virology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX, 75235, USA
SO J. Virol. (1995), 69(6), 3584-96
CODEN: JOVIAM; ISSN: 0022-538X
DT Journal
LA English
AB Human immunodeficiency virus type 1 (***HIV*** -1) gene expression is modulated by both viral and cellular factors. A regulatory element in the ***HIV*** -1 long terminal repeat known as TAR, which extends from nucleotides -18 to +80, is crit. for the activation of gene expression by the transactivator protein, Tat. RNA transcribed from TAR forms a stable stem-loop structure which serves as the ***binding*** site for both Tat and cellular factors. Although TAR RNA is crit. for Tat activation, the role that TAR DNA plays in regulating ***HIV*** -1 gene expression is not clear. Several studies have demonstrated that TAR DNA can ***bind*** cellular proteins, such as UBP-1/LBP-1, which repress ***HIV*** -1 gene expression and other factors which are involved in the generation of short, nonprocessive transcripts. In an attempt to characterize addnl. cellular factors that ***bind*** to TAR DNA, a .lambda.gt11 expression cloning strategy involving the use of a portion of TAR DNA extending from -18 to +28 to probe a HeLa cDNA library was used. We identified a cDNA, designated TAR DNA- ***binding*** protein (TDP-43), which encodes a cellular factor of 43 kDa that ***binds*** specifically to pyrimidine-rich motifs in TAR. Antibody to TDP-43 was used in gel retardation assays to demonstrate that endogenous TDP-43, present in HeLa nuclear ext., also bound to TAR DNA. Although TDP-43 bound strongly to ***double*** - ***stranded*** TAR DNA via its ribonucleoprotein protein- ***binding*** motifs, it did not ***bind*** to TAR RNA extending from +1 to +80. To det. the function of TDP-43 in regulating ***HIV*** -1 gene expression, in vitro transcription anal. was performed. TDP-43 repressed in vitro transcription from the ***HIV*** -1 long terminal repeat in both the presence and absence of Tat, but it did not repress transcription from other promoters such as the adenovirus major late promoter. In addn., transfection of a vector which expressed TDP-43 resulted in the repression of gene expression from an ***HIV*** -1 provirus. These results indicate that TDP-43 is capable of modulating both in vitro and in vivo ***HIV*** -1 gene expression by either altering or blocking the assembly

of transcription complexes that are capable of responding to Tat.

L9 ANSWER 7 OF 77 CA COPYRIGHT 1999 ACS
AN 123:190480 CA
TI Methods for isolation of most abundant oligonucleotides from complex mixtures
IN Beutel, Bruce A.; Coppola, George R.; Sherman, Michael I.; Cook, Alan F.; Fathi, Reza; Gao, Hetian; Rudolph, M. Jonathan; Bertelsen, Arthur H.
PA Pharmagenics, Inc., USA
SO PCT Int. Appl., 88 pp.
CODEN: PIXXD2
PI WO 9506751 A1 19950309
DS W: AU, CA, JP, US
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
AI WO 94-US9728 19940826
PRAI US 93-115470 19930901
DT Patent
LA English
AB The method of the present invention allows for screening of very large libraries of nucleic acids but does not require the reiterative PCR and ***binding*** steps customary in prior art methods. Instead there is only a single exposure to target followed by steps designed to identify those sequence that are most abundant in the selected mixt. Thus, ***double*** - ***stranded*** nucleic acids present in a mixt. thereof are converted to individual strands which are renatured under conditions which favor reannealing of the nucleic acids present at higher than av. concns. in the original mixt. The procedure can be used for identifying nucleic acids which ***bind*** to a target mol. or other compds. which ***bind*** to a target mol. (such as peptides or modified oligonucleotides) by using nucleic acids as a coding portion of a chimeric mol. which includes such compds. These chimeric mols. could be a combinatorial library comprising mols. contg. sep. target- ***binding*** and coding portions as described by Brenner and Lerner (Proc. Natl. Acad. Sci., 1992). A solid phase contg. a branched linker mol., one reactive group being protected with dimethoxytrityl and one with FMOC, was prepd. This modified matrix allows selective synthesis of, for example, an oligonucleotide on either arm of the linker. Such a matrix was used to prep. an RNA combinatorial library and the enrichment method of the invention was used to identify RNA mols. with high affinity for basic fibroblast growth factor.

L9 ANSWER 8 OF 77 CA COPYRIGHT 1999 ACS
AN 123:106196 CA
TI Calcium depletion from the endoplasmic reticulum activates the ***double*** - ***stranded*** RNA-dependent protein kinase (PKR) to

inhibit protein synthesis
AU Srivastava, Sri Prakash; Davies, Monique V.; Kaufman, Randal J.
CS Univ. Michigan Med. Cent., Howard Hughes Med. Inst., Ann Arbor, MI, 48105, USA
SO J. Biol. Chem. (1995), 270(28), 16619-24
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
AB Calcium depletion from the endoplasmic reticulum inhibits protein synthesis and correlates with increased phosphorylation of the .alpha. subunit of eukaryotic initiation factor 2 (eIF-2.alpha.) by a mechanism that does not require ongoing protein synthesis. To elucidate whether protein synthesis inhibition requires eIF-2.alpha. phosphorylation and whether eIF-2.alpha. phosphorylation is mediated by the ***double*** - ***stranded*** RNA-dependent protein kinase (PKR), the authors studied protein synthesis in response to calcium depletion mediated by calcium ionophore A23187 in cell lines overexpressing wild-type eIF-2.alpha., a mutant eIF-2.alpha. (S51A) that is resistant to phosphorylation, or a dominant neg. mutant PKR (K296P in catalytic subdomain II). Expression of either mutant eIF-2.alpha. or mutant PKR partially protected NIH3T3 cells from inhibition of protein synthesis upon A23187 treatment. In contrast, overexpression of wild-type PKR increased sensitivity to protein synthesis inhibition mediated by A23187 treatment. In a COS-1 monkey cell transient transfection system, increased eIF-2.alpha. phosphorylation in response to A23187 treatment was inhibited by expression of the dominant neg. PKR mutant. Overexpression of the PKR regulatory RNA ***binding*** domain, independent of the PKR catalytic domain, was sufficient to inhibit increased phosphorylation of eIF-2.alpha. upon A23187 treatment. In addn., over-expression of the ***HIV*** TAR RNA ***binding*** protein also inhibited eIF-2.alpha. phosphorylation upon A23187 treatment. Taken together, the data show that calcium depletion activates PKR to phosphorylate eIF-2.alpha., and this activation is likely mediated through the PKR RNA ***binding*** domain.

L9 ANSWER 9 OF 77 CA COPYRIGHT 1999 ACS
AN 123:106152 CA
TI Divalent cation modulation of the ribonuclease functions of human immunodeficiency virus reverse transcriptase
AU Cirino, Nick M.; Cameron, Craig E.; Smith, Jeffrey S.; Roth, Monica J.; Benkovic, Stephen J.; Le Grice, Stuart F. J.
CS School of Medicine, Case Western Reserve University, Cleveland, OH, 44106, USA
SO Biochemistry (1995), 34(31), 9936-43
CODEN: BICHAW; ISSN: 0006-2960
DT Journal

LA English
OS CJACS
AB The stimulatory effect of Mg2+ and Mn2+ on the RNase H functions of ***HIV*** -1 reverse transcriptase (RT) was evaluated using a model 90-nt RNA template/36-nt DNA primer. The wild-type enzyme exhibited similar endonuclease and directional processing activities in response to both cations, whereas RNase H* activity (hydrolysis of ***double*** - ***stranded*** RNA) was only evident in the presence of Mn2+. Enzyme altered at the p66 residue, Glu-478 (E478Q), which participates in metal ion ***binding*** , was completely inactive with Mg2+. However, Mn2+ specifically restored its endoribonuclease activity. In the presence of Mn2+, mutant RT also catalyzed specific removal of the tRNA replication primer, eliminating the possibility of contaminating Escherichia coli RNase H in the authors' recombinant enzyme. However, the efficiency with which mutant RT catalyzed transfer of nascent DNA between RNA templates (an event mandating RNase H activity) was severely reduced. These findings raise the possibility that directional processing activity is required to accelerate transfer of nascent DNA between templates during retroviral replication.

L9 ANSWER 10 OF 77 CA COPYRIGHT 1999 ACS
AN 123:76365 CA
TI Reduced replication of human immunodeficiency virus type 1 mutants that use reverse transcription primers other than the natural tRNA3Lys
AU Das, Atze T.; Klaver, Bep; Berkhout, Ben
CS Academic Medical Center, University of Amsterdam, Amsterdam, 1105 AZ, Neth.
SO J. Virol. (1995), 69(5), 3090-7
CODEN: JOVIAM; ISSN: 0022-538X
DT Journal
LA English
AB Replication of the human immunodeficiency virus type 1 (***HIV*** -1) and other retroviruses involves reverse transcription of the viral RNA genome into a ***double*** - ***stranded*** DNA. This reaction is primed by the cellular tRNA3Lys mol., which ***binds*** to a complementary sequence in the viral genome, referred to as the primer-***binding*** site (PBS). In order to study the specificity of primer usage, we constructed a set of ***HIV*** -1 mutants with altered PBS sites corresponding to other tRNA species (tRNAIle, tRNA1,2Lys, tRNAPhe, tRNAPro, tRNATrp). These mutant viruses were able to replicate, although with delayed replication kinetics compared with wild-type ***HIV*** -1. Identification of the tRNA species assocd. with the genomic RNA demonstrated ***binding*** of tRNAs complementary to the new PBS sites. However, the occupancy of the mutant PBS sites by these new primers was reduced and correlated well with the replication potential of

the mutant viruses. These results suggest that the PBS sequence is not sufficient for annealing of the tRNA primer. Upon prolonged culturing, all mutants reverted to the wild-type PBS3Lys sequence. Minor sequence changes in the nucleotide flanking the PBS site indicated that these reversions resulted from annealing of the wild-type tRNA3Lys primer onto the mutant PBS sites, followed by copying of part of the tRNA3Lys sequence during reverse transcription. Furthermore, the reversion efficiency of the different PBS mutants was found to correlate with their tRNA3Lys ***binding*** capacity. A remarkable reversion pathway was obsd. for the PBSPro variant (PBSPro .fwdarw. PBSIle .fwdarw. PBSwt). This pathway can be explained by efficient base pairing of tRNAIle to PBSPro, followed by annealing of tRNA3Lys onto the PBSIle intermediate. These results demonstrate that ***HIV*** -1 is dedicated to the tRNA3Lys primer and that factors other than the PBS sequence det. the selective primer usage of this retrovirus.

L9 ANSWER 11 OF 77 CA COPYRIGHT 1999 ACS
AN 123:76300 CA
TI The bend in RNA created by the trans-activation response element bulge of human immunodeficiency virus is straightened by arginine and by Tat-derived peptide
AU Zacharias, Martin; Hagerman, Paul J.
CS Dep. Biochemistry, Biophysics, and Genetics, Univ. Colorado Health Sciences, Denver, CO, 80262, USA
SO Proc. Natl. Acad. Sci. U. S. A. (1995), 92(13), 6052-6
CODEN: PNASA6; ISSN: 0027-8424
DT Journal
LA English
AB The trans-activation response element (TAR) found near the 5' end of the viral RNA of the human immunodeficiency virus contains a 3-nt bulge that is recognized by the virally encoded trans-activator protein (Tat), an important mediator of transcriptional activation. Insertion of the TAR bulge into ***double*** - ***stranded*** RNA is known to result in reduced electrophoretic mobility, suggestive of a bulge-induced bend. Furthermore, NMR studies indicate that Arg causes a change in the structure of the TAR bulge, possibly reducing the bulge angle. However, neither of these effects has been quantified, nor have they been compared with the effects of the TAR-Tat interaction. Recently, an approach for the quantification of bulge-induced bends has been described in which hydrodynamic measurements, employing the method of transient elec. birefringence, have yielded precise ests. for the angles of a series of RNA bulges, with the angles ranging from 7.degree. to 93.degree.. In the current study, transient elec. birefringence measurements indicate that the TAR bulge introduces a bend of 50.degree. .+-. 5.degree. in the absence of Mg2+. Addn. of Arg leads to essentially complete straightening

of the helix (to <10.degree.) with a transition midpoint in the 1 mM range. This transition demonstrates specificity for the TAR bulge: no comparable transition was obsd. for U3 or A3 (control) bulges with differing flanking sequences. An essentially identical structural transition is obsd. for the Tat-derived peptide, although the transition midpoint for the latter is near 1 .mu.M. Finally, low concns. of Mg2+ alone reduce the bend angle by .apprxeq.50%, consistent with the effects of Mg2+ on other pyrimidine bulges. This last observation is important in view of the fact that most previous structural/ ***binding*** studies were performed in the absence of Mg2+.

L9 ANSWER 12 OF 77 CA COPYRIGHT 1999 ACS
AN 122:285395 CA
TI Insights into DNA Polymerization Mechanisms from Structure and Function Analysis of ***HIV*** -1 Reverse Transcriptase
AU Patel, Premal H.; Jacobo-Molina, Alfredo; Ding, Jianping; Tantillo, Chris; Clark, Arthur D., Jr.; Raag, Reetta; Nanni, Raymond G.; Hughes, Stephen H.; Arnold, Edward
CS Center for Advanced Biotechnology and Medicine, Rutgers University, Piscataway, NJ, 08854-5638, USA
SO Biochemistry (1995), 34(16), 5351-63
CODEN: BICHAW; ISSN: 0006-2960
DT Journal
LA English
OS CJACS
AB When the single-stranded RNA genome of ***HIV*** -1 is copied into ***double*** - ***stranded*** DNA, the viral enzyme reverse transcriptase (RT) catalyzes the addn. of approx. 20 000 nucleotides; however, the precise mechanism of nucleotide addn. is unknown. In this study, the authors attempt to integrate the genetic data and biochem. mechanism of DNA polymn. with the structure of ***HIV*** -1 RT complexed with a dsDNA. The first step of polymn. involves the phys. assocn. of a polymerase with its nucleic acid substrate. A comparison of the structures of ***HIV*** -1 RT in the presence and absence of DNA indicates that the tip of the p66 thumb moves approx. 30 .ANG. upon DNA ***binding*** . This conformational change permits numerous interactions between residues of .alpha.-helixes H and I in the thumb subdomain and the DNA. Measurements of DNA ***binding*** affinity for nucleic acids with ***double*** - ***stranded*** DNAs that have an increasing no. of bases in the template overhang and mol. modeling suggest that portions of .beta.3 and .beta.4 within the finger subdomain ***bind*** single-stranded regions of the template. Measurements of nucleotide incorporation efficiency (kcat/Km) show that the ***binding*** and incorporation of the next complementary nucleotide are not dependent on the length of the template overhang. Mol. modeling of an incoming

nucleotide triphosphate (dTTP), based in part on the position of mercury atoms in a RT/DNA/Hg-UTP/Fab structure, suggests that portions of secondary structural elements .alpha.C-.beta.6, .alpha.E, .beta.11b, and .beta.9-.beta.10 det. the topol. of the dNTP- ***binding*** site. These results also suggest that nucleotide incorporation is accompanied by a protein conformational change that positions the dNTP for nucleophilic attack. Nucleophilic attack by the oxygen atom of the 3'-OH group of the primer strand could be metal-mediated, and Asp185 may be directly involved in stabilizing the transition state. The translocation step may be characterized by rotational as well as translational motions of ***HIV*** -1 RT relative to the DNA double helix. Some of the energy required for translocation could be provided by dNTP hydrolysis and could be coupled with conformational changes within the nucleic acid. A structural comparison of ***HIV*** -1 RT, Klenow fragment, and T7 RNA polymerase identified regions within T7 RNA polymerase which are not present in the other two polymerases that might help this polymerase to remain bound with nucleic acids and contribute to the ability of the T7 RNA polymerase to polymerize processively.

L9 ANSWER 13 OF 77 CA COPYRIGHT 1999 ACS
AN 122:283669 CA
TI Identification of a novel ***HIV*** -1 TAR RNA bulge ***binding*** protein
AU Baker, Bernadette; Muckenthaler, Martina; Vives, Eric; Blanchard, Andy; Braddock, Martin; Nacken, W.; Kingsman, Alan J.; Kingsman, Susan M.
CS Glaxo Group Res. Dev. Lts., Greenford/Midelesex, UB6 OHE, UK
SO Nucleic Acids Res. (1994), 22(16), 3365-72
CODEN: NARHAD; ISSN: 0305-1048
DT Journal
LA English
AB The Tat protein ***binds*** to TAR RNA to stimulate the expression of the human immunodeficiency virus type 1 (***HIV*** -1) genome. Tat is a 86 amino acid protein that contains a short region of basic residues (aa49-aa57) that are required for RNA ***binding*** and TAR is a 59 nucleotide stem-loop with a tripyrimidine bulge in the upper stem. TAR is located at the 5' end of all viral RNAs. In vitro, Tat specifically interacts with TAR by recognizing the sequence of the bulge and upper stem, with no requirement for the loop. However, in vivo the loop sequence is crit. for activation, implying a requirement for accessory cellular TAR RNA ***binding*** factors. A no. of TAR ***binding*** cellular factors have been identified in cell exts. and various models for the function of these factors have been suggested, including roles as coactivators and inhibitors. We have now identified a novel 38 kD cellular factor that has little general, single-stranded or ***double*** - ***stranded*** RNA ***binding*** activity, but that specifically

recognizes the bulge and upper stem region of TAR. The protein, referred to as BBP (bulge ***binding*** protein), is conserved in mammalian and amphibian cells and in Schizosaccharomyces pombe but is not found in Saccharomyces cerevisiae. BBP is an effective competitive inhibitor of Tat ***binding*** to TAR in vitro. Our data suggest that the bulge-stem recognition motif in TAR is used to mediate cellular factor/RNA interactions and indicates that Tat action might be inhibited by such competing reactions in vivo.

L9 ANSWER 14 OF 77 CA COPYRIGHT 1999 ACS
AN 122:233888 CA
TI ***Double*** - ***stranded*** peptide nucleic acids (PNA)
IN Buchardt, Ole; Egholm, Michael; Nielsen, Peter E.; Berg, Rolf; Norden, Benget; Wittung, Pernilla
PA Den.
SO PCT Int. Appl., 40 pp.
CODEN: PIXXD2
PI WO 9501369 A1 19950112
DS W: CA, JP
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
AI WO 94-IB211 19940701
PRAI US 93-88661 19930702
DT Patent
LA English
AB A novel class of compds., known as peptide nucleic acids, form ***double*** - ***stranded*** structures with one another and with ssDNA. The peptide nucleic acids generally comprise ligands such as naturally occurring DNA bases attached to a peptide backbone through a suitable linker such as amide.

L9 ANSWER 15 OF 77 CA COPYRIGHT 1999 ACS
AN 122:78522 CA
TI Regulation of the interferon-inducible eIF-2.alpha. protein kinase by small RNAs
AU Clemens, M. J.; Laing, K. G.; Jeffrey, I. W.; Schofield, A.; Sharp, T. V.; Elia, A.; Matys, V.; James, M. C.; Tilleray, V. J.
CS Dept. of Cellular and Molecular Sciences, Biochem. Div., St. George's Hospital Medical School, London, SW17 0RE, UK
SO Biochimie (1994), 76(8), 770-8
CODEN: BICMBE; ISSN: 0300-9084
DT Journal; General Review
LA English
AB This review with 92 refs. describes the structure and function of the ***double*** - ***stranded*** RNA-dependent protein kinase (PKR) and its interaction with RNA activators and inhibitors. The abilities of

small virally-encoded RNAs such as VA1 RNA of adenovirus, the Epstein-Barr virus encoded (EBER) RNAs, and the Tat-responsive region RNA of ***HIV*** -1 to ***bind*** to and regulate PKR are reviewed, and the physiol. implications of such regulation for the control of viral replication and cell growth are discussed. The potential effects on the activity of PKR of other proteins that ***bind*** ***double*** - ***stranded*** RNA and/or small viral and cellular RNAs are also considered.

L9 ANSWER 16 OF 77 CA COPYRIGHT 1999 ACS
AN 122:45697 CA
TI Inhibition of ***HIV*** -1 reverse transcriptase by defined template/primer DNA oligonucleotides: effect of template length and ***binding*** characteristics
AU Idriss, Haitham; Stammers, David K.
CS Department Molecular Sciences, Wellcome Research Laboratories, Kent, BR3 3BS, UK
SO J. Enzyme Inhib. (1994), 8(2), 97-112
CODEN: ENINEG; ISSN: 8755-5093
DT Journal
LA English
AB The interaction of partially ***double*** ***stranded*** DNA oligonucleotides with ***HIV*** -1 RT was studied by investigating their ability to inhibit the homopolymeric poly(rC) directed (dG) synthesis reaction. A 20/18 mer oligonucleotide, with a sequence based on the Lys3-tRNA primer region, showed stronger inhibition of the homopolymer RT reaction than a G/C rich oligonucleotide series lacking or possessing a hairpin moiety. Interaction of the enzyme with the G/C rich oligonucleotides, as detd. by IC50 measurements, was insensitive to the extent of the unpaired template region at the 3' or 5' position. Addn. of a hairpin moiety, composed of four thymidine bases, onto G/C rich oligonucleotides increase their inhibitory potency (at least six times) and shifted the mode of inhibition of RT to competitive with respect to poly(rC).(dG), which was otherwise mixed (competitive/noncompetitive) for the linear G/C rich and 20/18 mer oligonucleotides. The results indicate that interaction of the enzyme with the primer/template stem, but not with the unpaired template region, is an important step in complex formation.

L9 ANSWER 17 OF 77 CA COPYRIGHT 1999 ACS
AN 121:278715 CA
TI Direct interactions between autoantigen La and human immunodeficiency virus leader RNA
AU Chang, Yung-Nien; Kenan, Daniel J.; Keene, Jack D.; Gatignol, Anne; Jeang, Kuan-Teh
CS Laboratory of Molecular Microbiology, National Institute of Allergy and

Infectious Diseases, Bethesda, MD, 20892, USA
SO J. Virol. (1994), 68(11), 7008-20
CODEN: JOVIAM; ISSN: 0022-538X
DT Journal
LA English
AB We have characterized the in vivo and in vitro ***binding*** of human La protein to the human immunodeficiency virus type 1 (***HIV*** -1) leader RNA, the trans-activation response element (TAR). In immunopptn. studies using anti-La serum, La-TAR ribonucleoproteins were recovered from ***HIV*** -1-infected lymphocytes. Further characterization of this interaction revealed that La has preference for the TAR stem. However, TAR RNA recognition tolerated changes in the primary sequence of the stem as long as the secondary structure was conserved. This structural aspect of La-TAR recognition was confirmed in competition studies in which certain homopolymers influenced complex formation while other single-stranded and ***double*** - ***stranded*** RNAs had no effect. Deletion mutants of recombinant La protein were used to demonstrate that the residues responsible for ***binding*** to polymerase III precursor transcripts overlapped the ***binding*** domain for the TAR leader RNA. This finding of a direct interaction between La and TAR has functional implications for translational regulation of ***HIV*** -1 mRNAs as demonstrated in the accompanying report (Y. V. Svitkin, A. Pause, and N. Sonenberg, J. Virol. 68:7001-7007, 1994).

L9 ANSWER 18 OF 77 CA COPYRIGHT 1999 ACS
AN 121:273837 CA
TI Amino acid requirements of the nucleocapsid protein of ***HIV*** -1 for increasing catalytic activity of a Ki-ras ribozyme in vitro
AU Mueller, Gerd; Strack, Bettina; Dannull, Jens; Sproat, Brian S.; Surovoy, Andrej; Jung, Gunther; Moelling, Karin
CS Max-Planck-Inst. Molekulare Genetik, Berlin, D-14195, Germany
SO J. Mol. Biol. (1994), 242(4), 422-9
CODEN: JMOBAK; ISSN: 0022-2836
DT Journal
LA English
AB The nucleocapsid protein NCp7 of ***HIV*** -1 is a single-stranded nucleic acid ***binding*** protein with several functions such as specific recognition, dimerization and packaging of viral RNA, tRNA annealing to viral RNA and protection against nucleases. Since some of these functions involve annealing and ***double*** - ***stranded*** RNA-melting activity we applied the nucleocapsid protein to a hammerhead ribozyme specific for the activated Ki-ras mRNA in vitro, which carries at its mutated codon 12 a GUU site. A synthetic ribozyme contg. 2'-O-allyl-modified nucleotides and alternatively in vitro transcribed

ribozymes were used. At a one to one molar ratio of substrate to ribozyme almost no cleavage is obsd. at 37.degree.. Presence of a synthetic nucleocapsid protein significantly increases the catalytic activity of the ribozyme. Kinetic analyses by means of single and multiple turnover reactions performed at various substrate to ribozyme ratios lead to only a slight stimulation of the rate consts. for single turnover reactions. The rate consts. in multiple turnover reactions, however, are stimulated up to 17-fold by the presence of the nucleocapsid protein. The activating region of the nucleocapsid protein was characterized by a no. of mutants. The mutants demonstrate that activation requires both basic amino acid clusters as evidenced by point mutations. Deletion mutants indicate that the second zinc finger is totally dispensable and that replacement of the first zinc finger by a glycine-glycine spacer only slightly reduces the enhancing effect of the nucleocapsid protein on the ribozyme.

L9 ANSWER 19 OF 77 CA COPYRIGHT 1999 ACS
AN 121:197532 CA
TI Interaction of the Reverse Transcriptase of Human Immunodeficiency Virus Type 1 with DNA
AU Bakhanashvili, Mary; Hizi, Amnon
CS Sackler School of Medicine, Tel Aviv University, Tel Aviv, 69978, Israel
SO Biochemistry (1994), 33(40), 12222-8
CODEN: BICHAW; ISSN: 0006-2960
DT Journal
LA English
OS CJACS
AB During DNA synthesis, the ***binding*** of human immunodeficiency virus (***HIV***) reverse transcriptase (RT) to the template-primer precedes its ***binding*** to nucleotide triphosphates. The interaction of oligonucleotide DNA with ***HIV*** -1 RT was investigated by using a gel retardation assay. Both homodimeric (p66/p66) and heterodimeric (p66/p51) isoforms of ***HIV*** -1 RT were capable of ***binding*** the DNA oligomers. Thus, all further studies on the interaction of ***HIV*** -1 RT with DNA were done with heterodimeric RT. We have studied the conditions for optimal ***binding*** . The formation of the RT-DNA complex was primer-independent, and the extent of DNA ***binding*** was indistinguishable for both single-stranded and ***double*** - ***stranded*** DNA (either blunt-ended or recessed). The DNA ***binding*** activity of the RT was found to be dependent on oligonucleotide length. ***HIV*** -1 RT ***binds*** DNA with no apparent sequence specificity. Hence, this enzyme belongs to the sequence nonspecific DNA ***binding*** proteins. The interaction was found to be independent of DNA synthesis. The formation of the RT-DNA complex was not influenced by the presence of either template-complementary or noncomplementary dNTPs, indicating that neither DNA polymn. nor

binding of the RT to the dNTP affects the stability of the complex. The gel retardation assay was utilized to examine also the effect of various ***HIV*** -1 RT inhibitors (i.e., AZT-TP, ddTTP, TIBO, and 3,5,8-trihydroxy-4-quinolone) on the enzyme-DNA interaction. The results indicate differences in the modes of action of these compds. While there was a complete destabilization of the RT-DNA complex in the presence of 3,5,8-trihydroxy-4-quinolone, the addn. of AZT-TP, ddTTP, or TIBO had no apparent effect on the stability of the complex. Most effective anti- ***HIV*** compds. are inhibitors of ***HIV*** RT; hence the interaction of the enzyme with DNA might constitute a discrete step which can serve as a target for interference by novel specific anti- ***HIV*** RT drugs.

L9 ANSWER 20 OF 77 CA COPYRIGHT 1999 ACS
AN 121:150021 CA
TI IgMs produced by two acquired immune deficiency syndrome lymphoma cell lines: Ig ***binding*** specificity and VH-gene putative somatic mutation analysis
AU Ng, Valerie L.; Hurt, Mark H.; Fein, Charles L.; Khayam-Bashi, Farzad; Marsh, Jane; Nunes, Wylla M.; McPhaul, Laron W.; Feigal, Ellen; Nelson, Patricia; et al.
CS Sch. Med., Univ. California, San Francisco, CA, USA
SO Blood (1994), 83(4), 1067-78
CODEN: BLOOAW; ISSN: 0006-4971
DT Journal
LA English
AB Two B-cell lines, 2F7 and 10C9, were established by single cell cloning from biopsies obtained from two acquired immune deficiency syndrome patients with Burkitt's lymphoma. Representation of the original tumors was verified by demonstration of (1) identical biallelic rearrangement of Ig genes for 2F7 and (2) shared idiotype for 10C9. Both cell lines displayed cell-surface Ig and secreted Ig (IgM .lambda. for 2F7, IgM .kappa. for 10C9). IgMs from both cell lines immunopptd. actin; in addn. 2F7 IgM .lambda. immunopptd. recombinant human immunodeficiency virus type 1 (***HIV*** -1) gp160.2F7 IgM .lambda. did not react with other autoantigens (***double*** - ***stranded*** and single-stranded DNA, actin, bovine serum albumin, IgG), whereas 10C9 IgM .kappa. reacted with human IgG. The 2F7 IgM heavy-chain variable region (VH) showed a 95% nucleotide homol. with a previously sequenced VHIII germline gene, hv3019b9, whereas the 10C9 IgM VH showed a 95% homol. with a previously sequenced VHIV germline gene, VH4.21. Use of minimally modified VH genes and demonstration of reactivity with chronically present antigens (ie, actin ***HIV*** -1 gp160, or human IgG) suggests that B cells in ***HIV*** -1-infected individuals proliferating in response to chronic antigenic stimulation may be at increased risk for lymphomagenesis.

L9 ANSWER 21 OF 77 CA COPYRIGHT 1999 ACS
AN 121:79933 CA
TI TAR RNA- ***binding*** protein is an inhibitor of the interferon-induced protein kinase PKR
AU Park, Heesung; Davies, Monique V.; Langland, Jeffrey O.; Chang, Hwai Wen; Nam, Yong Suk; Tartaglia, James; Paoletti, Enzo; Jacobs, Bertram L.; Kaufman, Randal J.; Venkatesan, Sundararajan
CS Lab. Mol. Microbiol., Natl. Inst. Allergy Infect. Dis., Bethesda, MD, 20892, USA
SO Proc. Natl. Acad. Sci. U. S. A. (1994), 91(11), 4713-17
CODEN: PNASA6; ISSN: 0027-8424
DT Journal
LA English
AB A cDNA encoding a ***double*** - ***stranded*** -RNA (dsRNA)-***binding*** protein was isolated by screening a HeLa cell cDNA-expression library for proteins that ***bind*** the ***HIV*** -1 Rev-responsive-element RNA. The cDNA encoded a protein that was identical to TRBP, the previously reported cellular protein that ***binds*** the transactivation response element (TAR) RNA of human immunodeficiency virus type 1. TRBP inhibited phosphorylation of the interferon-induced ribosome-assocd. protein kinase PKR and of the eukaryotic translation initiation factor eIF-2.alpha. in a transient-expression system in which the translation of a reporter gene was inhibited by the localized activation of PKR. TRBP expression in HeLa cells complemented the growth and protein-synthesis defect of a vaccinia virus mutant lacking the expression of the dsRNA- ***binding*** protein E3L. These results implicate TRBP as a cellular regulatory protein that ***binds*** RNAs contg. specific secondary structure(s) to mediate the inhibition of PKR activation and stimulate translation in a localized manner.

L9 ANSWER 22 OF 77 CA COPYRIGHT 1999 ACS
AN 121:26350 CA
TI Sensitivity of wild-type human immunodeficiency virus type 1 reverse transcriptase to dideoxynucleotides depends on template length; the sensitivity of drug-resistant mutants does not
AU Boyer, Paul L.; Tantillo, Chris; Jacobo-Molina, Alfredo; Nanni, Raymond G.; Ding, Jianping; Arnold, Edward; Hughes, Stephen H.
CS Frederick Cancer Res. Dev. Cent., Natl. Cancer Inst., Frederick, MD, 21702-1201, USA
SO Proc. Natl. Acad. Sci. U. S. A. (1994), 91(11), 4882-6
CODEN: PNASA6; ISSN: 0027-8424
DT Journal
LA English

AB Anal. of the three-dimensional structure of human immunodeficiency virus type 1 (***HIV*** -1) reverse transcriptase (RT) complexed with ***double*** - ***stranded*** DNA indicates that while many nucleoside-resistance mutations are not at the putative dNTP ***binding*** site, several are in positions to interact with the template-primer. Wild-type ***HIV*** -1 RT and two nucleoside-resistant variants, Leu74 .fwdarw. Val and Glu89 .fwdarw. Gly, have been analyzed to det. the basis of resistance. The ability of the wild-type enzyme to incorporate, or reject, a 2',3'-dideoxynucleoside triphosphate (ddNTP) is strongly affected by interactions that take place between the enzyme and the extended template strand 3-6 nt beyond the polymerase active site. Inspection of a model of the enzyme with an extended template suggests that this interaction involves the fingers sub-domain of the p66 subunit in the vicinity of Leu74. These data provide direct evidence that the fingers sub-domain of the p66 subunit of ***HIV*** -1 RT interacts with the template strand. The wild-type enzyme is resistant to ddITP if the template extension is 3 nt or less and becomes sensitive only when the template extends more than 3 or 4 nt beyond the end of the primer strand. However, the mutant enzymes are resistant with both short and long template extensions. Taken together with the three-dimensional structure of ***HIV*** -1 RT in complex with ***double*** - ***stranded*** DNA, these data suggest that resistance to the dideoxynucleotide inhibitors results from a repositioning or change in the conformation of the template-primer that alters the ability of the enzyme to select or reject an incoming dNTP.

L9 ANSWER 23 OF 77 CA COPYRIGHT 1999 ACS
AN 120:315191 CA
TI Inhibition of DNA topoisomerase I activity by 2',5'-oligoadenylates and mismatched ***double*** - ***stranded*** RNA in uninfected and ***HIV*** -1-infected H9 cells
AU Schroeder, Heinz C.; Kelve, Merike; Schaecke, Heike; Pfleiderer, Wolfgang; Charubala, Ramamurthy; Suhadolnik, Robert J.; Mueller, Werner E. G.
CS Inst. Physiol. Chem., Johannes Gutenberg-Univ., Mainz, 55099, Germany
SO Chem.-Biol. Interact. (1994), 90(2), 169-83
CODEN: CBINA8; ISSN: 0009-2797
DT Journal
LA English
AB 2',5'-Oligoadenylates (2-5As) inhibit the type I DNA topoisomerase activity both in uninfected and ***HIV*** -1-infected human T cell line H9 as well as the purified enzyme (calf thymus). Topoisomerase I activity was detd. by measuring the relaxation of neg. supercoiled pBR322 DNA. Inhibition of topoisomerase I by 2-5A depends on the chain length of the oligomer and the presence of 5' phosphate. The 5'-triphosphate of the 2-5A hexamer was most active (almost total inhibition of DNA relaxation at

10 .mu.M concn.); the 2-5A core trimer (at 100 .mu.M) displayed no significant effect. In crosslinking and immunopptg. expts. the authors present evidence that 2-5A (32P-labeled 2-5A deriv., ppp(A2'p)2 A[32P]pC) is able to ***bind*** to nuclear topoisomerase I. The mismatched dsRNA, poly(I).cntdot.poly(C12U) (Ampligen), exhibited a strong anti-***HIV*** -1 activity. However, the authors' data show that this antiviral effect is not related to topoisomerase I inhibition. On the other hand, the authors did observe the prodn. of longer oligomers of 2-5A in cells treated with poly(I).cntdot.poly(C12U). It remains speculative, whether the in vivo effect could be catalyzed by this activity of poly(I).cntdot.poly(C12U). In addn. the authors showed that 2-5A also inhibits topoisomerase I activity assocd. with isolated ***HIV*** -1 particles.

L9 ANSWER 24 OF 77 CA COPYRIGHT 1999 ACS
AN 120:292249 CA
TI Triple Helix Formation with Short Oligonucleotide-Intercalator Conjugates Matching the ***HIV*** -1 U3 LTR End Sequence
AU Mouscadet, Jean-Francois; Ketterle, Christophe; Goulaouic, Helene; Carteau, Sandrine; Subra, Frederic; Le Bret, Marc; Auclair, Christian
CS Laboratoire de Physicochimie et de Pharmacologie des Macromolecules Biologiques, Institut Gustave-Roussy, Villejuif, 94805, Fr.
SO Biochemistry (1994), 33(14), 4187-96
CODEN: BICHAW; ISSN: 0006-2960
DT Journal
LA English
OS CJACS
AB In an attempt to target short purine sequences in view of pharmacol. application, the authors have synthesized three new TFO (triple-helix-forming oligonucleotide) conjugates in which an intercalating oxazolopyridocarbazole (OPC) chromophore is linked by a pentamethylene linker to a 7-mer oligonucleotide matching the polypurine/polypyrimidine sequence located in the ***HIV*** -1 U3 LTR end region. The TFO moiety of conjugates are 5'CCTTCCC, 5'GGGAAGG, and 5'GGGTTGG. Their ability to ***bind*** to ***double*** -***stranded*** DNA targets was examd. This ***binding*** is demonstrated by a footprinting technique using DNase I as a cleaving agent. The complex involved intermol. pyr-pur*pyr or pur-pur*pyr triple helix. Pyrimidine TFO-OPC ***binds*** in a pH-dependent manner, whereas the others do not. The formation of the complex has been investigated at neutral pH and increasing temp. The protection due to the purine and mixed TFO-OPC was pH independent and remained identical up to 40 .degree.C. To det. the position of the OPC chromophore, mol. modeling was undertaken on the purine-conjugate/target complex. It has been suggested that the complex involved the intercalation of the OPC at the

triplex-duplex junction with a small unwinding at the next excluded site.

L9 ANSWER 25 OF 77 CA COPYRIGHT 1999 ACS
AN 120:235483 CA
TI Synergistic anti-human immunodeficiency viral (***HIV*** -1) effect of the immunomodulator Ampligen (mismatched ***double*** - ***stranded*** RNA) with inhibitors of reverse transcriptase and ***HIV*** -1 regulatory proteins
AU Ushijima, H.; Tsiapalis, C. M.; Daum, T.; Schroeder, H. C.; Matthes, E.; Engels, J. W.; Mag, M.; Muth, J.; Mueller, W. E. G.
CS AIDS Res. Cent., Natl. Inst. Health, Musashimurayama, 208, Japan
SO Antiviral Chem. Chemother. (1993), 4(6), 315-21
CODEN: ACCHEH; ISSN: 0956-3202
DT Journal
LA English
AB The potent antiviral effect of ***double*** ***stranded*** RNA, such as the mismatched poly(I).cntdot.poly(C12U) [Ampligen], 2',3'-dideoxy-3'-fluorothymidine (FddThd) and antisense oligodeoxynucleotides (ODN) has been established in in vitro systems using cells infected with the human immunodeficiency virus type 1 (***HIV*** -1). The authors report here that the immunomodulator poly(I).cntdot.poly(C12U) interacts synergistically with (1) the reverse transcriptase inhibitor FddThd (FIC value: 0.43), (2) the modified (5'- and 3'-end capped thioates) antisense ODN-4 directed against the splice acceptor site of the ***HIV*** -1/tat gene (FIC value: 0.66) and (3) also with pyronin Y, a compd. which prevents ***binding*** of ***HIV*** -1 Rev protein to the ***HIV*** -1 RRE element. These data suggest that combinations of poly(I).cntdot.poly(C12U), a stimulator of the natural antiviral protection system of the cells, with compds. targeting HIV1-specific processes should be considered as candidate treatments of AIDS patients.

L9 ANSWER 26 OF 77 CA COPYRIGHT 1999 ACS
AN 120:208170 CA
TI Inhibition of entry of ***HIV*** into cells by poly(A).cntdot.poly(U)
AU Krust, Bernard; Callebaut, Christian; Hovanessian, Ara G.
CS Unite Virol. Immunol. Cell., Inst. Pasteur, Paris, 75724, Fr.
SO AIDS Res. Hum. Retroviruses (1993), 9(11), 1087-90
CODEN: ARHRE7; ISSN: 0889-2229
DT Journal
LA English
AB Polyadenylic-polyuridylic acid [poly(A).cntdot.poly(U)] is a synthetic ***double*** - ***stranded*** RNA that has been shown to manifest both antitumoral and immunomodulatory activities. Previously, it was reported that poly(A).cntdot.poly(U) inhibits ***HIV*** infection in cell

cultures. Here direct evidence is provided to demonstrate that the inhibitory action of poly(A).cntdot.poly(U) is through its capacity to prevent entry of ***HIV*** particles into CD4-pos. T lymphocytes. Such inhibition of ***HIV*** entry is also obsd. in the case of other polyanions such as heparin, dextran sulfate, and poly(I).cntdot.poly(C). The mechanism of inhibition appears to occur postbinding of ***HIV*** particles to the CD4 receptor mols., because the ***binding*** of the external envelope glycoprotein of ***HIV*** -1 (gp120) is not affected significantly in the presence of poly(A).cntdot.poly(U) or other polyanions. These results confirm the potential of poly(A).cntdot.poly(U) as an antiviral drug against ***HIV*** infection.

L9 ANSWER 27 OF 77 CA COPYRIGHT 1999 ACS
AN 120:184602 CA
TI Mutagenicity and pausing of ***HIV*** reverse transcriptase during ***HIV*** plus-strand DNA synthesis
AU Ji, Jiuping; Hoffmann, Jean Sebastien; Loeb, Lawrence
CS Dep. Pathol., Univ. Washington, Seattle, WA, 98195, USA
SO Nucleic Acids Res. (1994), 22(1), 47-52
CODEN: NARHAD; ISSN: 0305-1048
DT Journal
LA English
AB The unusually high frequency of misincorporation by ***HIV*** -1 reverse transcriptase (***HIV*** RT) is likely to be the major factor in the rapid accumulation of viral mutations in AIDS, esp. in the env gene. To investigate the ability of ***HIV*** RT to copy the env gene, the authors subcloned an ***HIV*** env gene fragment into a single-stranded DNA vector and measured the progression of synthesis by ***HIV*** RT. The ***HIV*** RT, but not RT from avian myeloblastosis virus, DAN polymerase-.alpha. or T7 DNA polymerase, pauses specifically at polydeoxy-adenosine stretches within the env gene. The frequency of bypassing the polyadenosine stretches by ***HIV*** RT is enhanced by increasing the ratio of enzyme to template. The authors measured the fidelity of DNA synthesis within a segment of the hypervariable region 1 of the env gene (V-1) contg. a polydeoxyadenosine sequence by repetitively copying the DNA by ***HIV*** RT, and then cloning and sequencing the copied fragments. The authors found that 27% of the errors identified in V-1 sequence were frameshift mutations opposite the polyadenosine tract, a site where strong pausing was obsd. Pausing of ***HIV*** RT at the polyadenosine tract could be enhanced by either distamycin A or netropsin, (A-T)-rich minor groove ***binding*** peptides. Moreover, netropsin increases the frequency of frameshift mutations in expts. in which ***HIV*** RT catalyzes gap filling synthesis within the lacZ gene in ***double*** - ***stranded*** circular M13mp2 DNA. The results suggest that the

enhanced mutation frequency may be due to increased pausing at netropsin-modified polyadenosine tracts. Therefore, netropsin and related A-T ***binding*** chems. may selectively enhance frameshift mutagenesis induced by ***HIV*** RT and yield predominantly non-viable virus.

L9 ANSWER 28 OF 77 CA COPYRIGHT 1999 ACS
AN 120:155159 CA
TI HIV1 integrase expressed in Escherichia coli from a synthetic gene
AU Holler, Tod P.; Foltin, Susan K.; Ye, Qi Zhuang; Hupe, Donald J.
CS Dep. Biochem., Parke-Davis Pharm. Res., Ann Arbor, MI, 48105, USA
SO Gene (1993), 136(1-2), 323-8
CODEN: GENED6; ISSN: 0378-1119
DT Journal
LA English
AB Human immunodeficiency virus type 1 (HIV1) integrase is cleaved from the gag-pol precursor by the HIV1 protease. The resulting 32-kDa protein is used by the infecting virus to insert a linear, ***double*** - ***stranded*** DNA copy of its genome, prepd. by reverse transcription of viral RNA, into the host cell's chromosomal DNA. In order to achieve high levels of expression, to minimize an internal initiation problem and to facilitate mutagenesis, the authors have designed and synthesized a gene encoding the integrase from the infectious mol. clone, pNL4-3. Codon usage was optimized for expression in Escherichia coli and unique restriction sites were incorporated throughout the gene. A 905-bp cassette contg. a ribosome- ***binding*** site, a start codon and the integrase-coding sequence, sandwiched between EcoRI and HindIII sites, was synthesized by overlap extension of nine long synthetic oligodeoxyribonucleotide [90-120 nucleotides (nt)] and subsequent amplification using two primers (28-30 nt). The cassette was sub-cloned into the vector pKK223-3 for expression under control of a tax promoter. The protein produced from this highly expressed gene has the expected N-terminal sequence and mol. mass, and displays the DNA processing, DNA joining and disintegration activities expected from recombinant integrase. These studies have demonstrated the utility of codon optimization, and lay the groundwork for structure-function studies of HIV1 integrase.

L9 ANSWER 29 OF 77 CA COPYRIGHT 1999 ACS
AN 120:126830 CA
TI Adeno-associated virus type 2 rep gene-mediated inhibition of basal gene expression of human immunodeficiency virus type 1 involves its negative regulatory functions
AU Oelze, Ingo; Rittner, Karola; Sczakiel, Georg
CS Forschungschwerpunkt Angew. Tumorvirol., Dtsch. Krebsforschungszent., Heidelberg, D-69120, Germany

SO J. Virol. (1994), 68(2), 1229-33
CODEN: JOVIAM; ISSN: 0022-538X
DT Journal
LA English
AB Adeno-assocd. virus type 2 (AAV-2), a human parvovirus which is apathogenic in adults, inhibits replication and gene expression of human immunodeficiency virus type 1 (***HIV*** -1) in human cells. The rep gene of AAV-2, which was shown earlier to be sufficient for this neg. interference, also down-regulated the expression of heterologous sequences driven by the long terminal repeat (LTR) of ***HIV*** -1. This effect was obsd. in the absence of the ***HIV*** -1 transactivator Tat, i.e., at basal levels of LTR-driven transcription. Here, the authors studied the involvement of functional subsequences of the ***HIV*** -1 LTR in rep-mediated inhibition in the absence of Tat. Mutated LTRs driving an indicator gene (cat) were cointroduced into human SW480 cells together with rep alone or with ***double*** - ***stranded*** DNA fragments of RNA contg. sequences of the ***HIV*** -1 LTR. The results indicate that rep strongly enhances the function of neg. regulatory elements of the LTR. In addn., the expts. revealed a transcribed sequence element located within the TAR-coding sequence termed AHHH (AAV- ***HIV*** homol. element derived from ***HIV*** -1) which is involved in rep-mediated inhibition. The AHHH element is also involved in down-regulation of basal expression levels in the absence of rep, suggesting that AHHH also contributes to neg. regulatory functions of the LTR of ***HIV*** -1. In contrast, pos. regulatory elements of the ***HIV*** -1 LTR such as the NF.kappa.B and SP1 ***binding*** sites have no significant influence on th rep-mediated inhibition.

L9 ANSWER 30 OF 77 CA COPYRIGHT 1999 ACS
AN 120:23569 CA
TI Triple helix recognition of DNA
IN Dervan, Peter B.
PA California Institute of Technology, USA
SO PCT Int. Appl., 42 pp.
CODEN: PIXXD2
PI WO 9318187 A1 19930916
DS W: CA, JP
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
AI WO 93-US2352 19930311
PRAI US 92-850503 19920313
DT Patent
LA English
AB Described are oligonucleotides and processes for their use for specific recognition of a target sequence in ***double*** - ***stranded*** nucleic acid through the formation of a triple helix. The

oligonucleotides contain nebularine (N) and ***bind*** to one of the strands of the target sequence. The oligonucleotides can be used as diagnostic or therapeutic agents through incorporation of an appropriate group in .gtoreq.1 nucleotides in the triple helix-forming oligonucleotide. The purine-rich target sequence 5'-d(AGAG2CGAG4CG2)-3'.bul.5'-d(C2GC4TCGC2TCT)-3', a sequence occurring in the ***HIV*** genome, was prepd. with plasmid pULHIV by recombinant methods. A restriction fragment contg. the target sequence was reacted with 5'-d(G2Z2G4Z1GZ2G2Z1GT*)-3' [Z1 = A, T, N; Z2 = N, A, G, C, T; T* = thymidine-EDTA.bul.Fe(II)] (I) in the presence of dithiothreitol at 37.degree.. One major cleavage product indicated sequence-specific cleavage was only obsd. for 4 of I.

L9 ANSWER 31 OF 77 CA COPYRIGHT 1999 ACS
AN 119:242692 CA
TI A cluster of strong topoisomerase II cleavage sites is located near an integrated human immunodeficiency virus
AU Howard, Michael T.; Griffith, Jack D.
CS Lineberger Compr. Cancer Cent., Univ. North Carolina, Chapel Hill, NC, 27599, USA
SO J. Mol. Biol. (1993), 232(4), 1060-8
CODEN: JMOBAK; ISSN: 0022-2836
DT Journal
LA English
AB The human immunodeficiency virus (***HIV***) integrates into host cellular DNA as a ***double*** ***strand*** DNA mol. Here a previously studied ***HIV*** isolate was examd. for ***binding*** and cleavage by topoisomerase II in vitro within the 5' LTR region and human flanking DNA. A cluster of strong ***binding*** and cleavage sites in the human sequences was located approx. 850 bp upstream from the integration site. This region maps to a locus consisting of a complex repeating element, and alternating purine/pyrimidine sequences. Topoisomerase II ***binding*** and cleavage sites were also located within the ***HIV*** 5' LTR, in particular a site overlying the DNA sequence coding for TAR, another inverted repeat element in the DNA.

L9 ANSWER 32 OF 77 CA COPYRIGHT 1999 ACS
AN 119:218972 CA
TI Design and synthesis of RNA miniduplexes via a synthetic linker approach. 2. Generation of covalently closed, ***double*** - ***stranded*** cyclic ***HIV*** -1 TAR RNA analogs with high Tat- ***binding*** affinity
AU Ma, Michael Y. X.; McCallum, Kirk; Climie, Shane C.; Kuperman, Raya; Lin, Wing C.; Sumner-Smith, Martin; Barnett, Richard W.
CS Allelix Biopharm. Inc., Mississauga, ON, L4V 1P1, Can.

SO Nucleic Acids Res. (1993), 21(11), 2585-9
CODEN: NARHAD; ISSN: 0305-1048
DT Journal
LA English
AB An approach was recently developed which allows rapid generation of short, ***double*** - ***stranded*** oligonucleotides whereby one end of the duplex was joined and stabilized by a synthetic linker of specific design (miniduplexes). Model miniduplexes based on the ***HIV*** -1 TAR RNA hairpin are thermodynamically stable and good substrates for ***binding*** by the ***HIV*** -1 Tat protein which normally ***bind*** to natural TAR. These studies were now extended to the design, synthesis, and anal. of the ***binding*** properties of covalently closed, ***double*** - ***stranded*** , cyclic RNA miniduplexes. A strategy using automated chem. synthesis and T4 RNA ligase-catalyzed cyclization was employed to generate cyclic oligoribonucleotides. When both ends of a shortened, wild-type TAR RNA stem (9 bp) were covalently linked through either nucleotidic loops (4-6 nt) or synthetic linkers (derivatized from hexaethylene glycol), the resulting cyclic TAR RNA analogs were good substrates for ***binding*** by both Tat-derived peptide or full-length Tat protein. Interestingly, the cyclic TAR analogs failed to show any ***binding*** if the synthetic linker was reduced in length (e.g. derivatized from triethylene glycol), although such linkers are acceptable in the hairpin-shaped miniduplexes series. This implies that RNA conformational changes are required for Tat ***binding*** and that these changes are restricted in certain cyclic variants. These findings suggest that covalently-closed nucleic acid miniduplexes may be useful both to study nucleic acid-protein interactions as well as to provide a basis for therapeutic intervention as transcription decoys.

L9 ANSWER 33 OF 77 CA COPYRIGHT 1999 ACS
AN 119:203715 CA
TI Design and synthesis of RNA miniduplexes via a synthetic linker approach
AU Ma, Michael Y. X.; Reid, Lorne S.; Climie, Shane C.; Lin, Wing C.; Kuperman, Raya; Sumner-Smith, Martin; Barnett, Richard W.
CS Allelix Biopharm. Inc., Mississauga, ON, L4V 1P1, Can.
SO Biochemistry (1993), 32(7), 1751-8
CODEN: BICHAW; ISSN: 0006-2960
DT Journal
LA English
OS CJACS
AB ***Double*** - ***stranded*** oligodeoxyribonucleotides or single-stranded oligoribonucleotides with specific secondary structure have been proposed as potential antagonists to target nucleic acid-***binding*** proteins (the sense approach). A major limitation of this

strategy is that these derivs. are generally considered to be too large for pharmaceutical applications. Using a synthetic linker approach, nucleic acid duplexes of a much smaller size (miniduplexes) were generated directly by a std. oligonucleotide synthesis. In this approach, four synthetic linkers were incorporated into GGAGAUCUGAGCOP(O)(O-)OXOP(O)(O-)OGCUCUCUCC [I, X = (CH2)9, (CH2CH2O)2CH2CH2, (CH2)3, (CH2CH2O)5CH2CH2, (CH2CH2O)3P(O)(O-)O(CH2CH2O)2CH2CH2,(CH2)3OP(O)(O-)O(CH2)3], a model RNA mol. based on the TAR stem-loop structure of ***HIV*** -1. Their thermal stabilities were evaluated by measuring denaturation profiles (Tm measurements). I were then assessed for their ability to ***bind*** to either a full-length protein (***HIV*** -1 Tat protein) or a short peptide (Tat-derived peptide) through RNA mobility shift assays. Results from this study indicate that such modified miniduplex structures retain full ***binding*** activity relative to that of the wild-type sequence (Kd values), while Tm values were increased by 24-31.degree. compared to an open duplex of the same length. This system provides a new direction in the use of nucleic acid miniduplexes as a novel class of oligonucleotide analogs for both fundamental research and possible therapeutic applications.

L9 ANSWER 34 OF 77 CA COPYRIGHT 1999 ACS
AN 119:198040 CA
TI Structure of a new nucleic-acid- ***binding*** motif in eukaryotic transcriptional elongation factor TFIIS
AU Qian, Xiuqu; Jeon, Choonju; Yoon, HoSup; Agarwal, Kan; Weiss, Michael A.
CS Dep. Biol. Chem. Mol. Pharmacol., Harvard Med. Sch., Boston, MA, 02115, USA
SO Nature (London) (1993), 365(6443), 277-9
CODEN: NATUAS; ISSN: 0028-0836
DT Journal
LA English
AB Transcriptional elongation involves dynamic interactions among RNA polymerase and single-std. and ***double*** - ***stranded*** nucleic acids in the ternary complex. In prokaryotes its regulation provides an important mechanism of genetic control. Analogous eukaryotic mechanisms are not well understood, but may control expression of proto-oncogenes and viruses, including the human immunodeficiency virus ***HIV*** -1. The highly conserved eukaryotic transcriptional elongation factor TFIIS enables RNA polymerase II (RNAPII) to read through pause or termination sites, nucleosomes and sequence-specific DNA- ***binding*** proteins. Two distinct domains of human TFIIS, which ***bind*** RNAPII and nucleic acids, regulate read-through and possibly nascent transcript cleavage. Here, the three-dimensional NMR structure of a Cys4 nucleic-acid- ***binding*** domain from human TFIIS is described. Unlike previously characterized zinc modules, which contain an

.alpha.-helix, this structure consists of a three-stranded .beta.-sheet. Analogous Cys4 structural motifs may occur in other proteins involved in DNA or RNA transactions, including RNAPII itself. This new structure, designated the Zn ribbon, extends the repertoire of Zn-mediated peptide architectures and highlights the growing recognition of the .beta.-sheet as a motif of nucleic-acid recognition.

L9 ANSWER 35 OF 77 CA COPYRIGHT 1999 ACS
AN 119:134365 CA
TI Crystal structure of bacteriophage T7 RNA polymerase at 3.3 .ANG. resolution
AU Sousa, Rui; Chung, Yong Je; Rose, John P.; Wang, Bi Cheng
CS Dep. Biol. Sci., Univ. Pittsburgh, Pittsburgh, PA, 15260, USA
SO Nature (London) (1993), 364(6438), 593-9
CODEN: NATUAS; ISSN: 0028-0836
DT Journal
LA English
AB The crystal structure of T7 polymerase reveals a mol. organized around a cleft that can accommodate a ***double*** - ***stranded*** DNA template. A portion (.apprx. 45%) of the mol. displays extensive homol. to the polymerase domain of Klenow fragment and more limited homol. to the human immunodeficiency virus ***HIV*** -1 reverse transcriptase. A comparison of the structures and sequences of these polymerases identifies structural elements that may be responsible for discriminating between ribonucleotide and deoxyribonucleotide substrates, and RNA and DNA templates. The relative locations of the catalytic site and a specific promoter recognition residue allow the orientation of the polymerase on the template to be defined.

L9 ANSWER 36 OF 77 CA COPYRIGHT 1999 ACS
AN 119:112206 CA
TI Crystal structure of human immunodeficiency virus type 1 reverse transcriptase complexed with ***double*** - ***stranded*** DNA at 3.0 .ANG. resolution shows bent DNA
AU Jacobo-Molina, Alfredo; Ding, Jianping; Nanni, Raymond G.; Clark, Arthur D., Jr.; Lu, Xiaode; Tantillo, Chris; Williams, Roger L.; Kamer, Greg; Ferris, Andrea L.; et al.
CS Cent. Adv. Biotechnol. Med., Rutgers Univ., Piscataway, NJ, 08854-5638, USA
SO Proc. Natl. Acad. Sci. U. S. A. (1993), 90(13), 6320-4
CODEN: PNASA6; ISSN: 0027-8424
DT Journal
LA English
AB The crystal structure of a ternary complex of human immunodeficiency virus type 1 reverse transcriptase (***HIV***. -1RT) heterodimer (p66/p51), a

19-base/18-base ***double*** - ***stranded*** DNA template-primer, and a monoclonal antibody Fab fragment has been detd. at 3.0 .ANG. resoln. The four individual subdomains of RT that make up the polymerase domains of p66 and p51 are named fingers, palm, thumb, and connection (Kohlstaedt, L. A., et al., 1992). The overall folding of the subdomains is similar in p66 and p51 but the spatial arrangements of the subdomains are dramatically different. The template-primer has A-form and B-form regions sepd. by a significant bend (40-45.degree.). The most numerous nucleic acid interactions with protein occur primarily along the sugar-phosphate backbone of the DNA and involve amino acid residues of the palm, thumb, and fingers of p66. Highly conserved regions are located in the p66 palm near the polymerase active site. These structural elements, together with two .alpha.-helixes of the thumb of p66, act as a clamp to position the template-primer relative to the polymerase active site. The 3'-hydroxyl of the primer terminus is close to the catalytically essential Asp-110, Asp-185, and Asp-186 residues at the active site and is in a position for nucleophilic attack on the .alpha.-phosphate of an incoming nucleoside triphosphate.

L9 ANSWER 37 OF 77 CA COPYRIGHT 1999 ACS
AN 119:64906 CA
TI Generation and selection of novel DNA- ***binding*** proteins
IN Ladner, Robert C.; Guterman, Sonia K.; Kent, Rachel B.; Ley, Arthur C.
PA Protein Engineering Corp., USA
SO U.S., 145 pp. Cont.-in-part of U.S. 5,096,815.
CODEN: USXXAM
PI US 5198346 A 19930330
AI US 90-558011 19900726
PRAI US 89-293980 19890106
DT Patent
LA English
AB A method for selecting a pair of genes encoding proteins that ***bind*** to homooligomeric DNA (DBPs) and that assoc. to form a hybrid hetero-oligomeric protein that ***binds*** to a predetd. nonpalindromic, ***double*** - ***stranded*** DNA target sequence is described. The genes are selected in cells that have first been transformed with a selection vector contg. 2 operons, each operon contg. a promoter, a target sequence, and a selectable or screenable gene. These cells are also transformed with a 2nd vector contg. a DBP gene that has been mutagenized by a non-specific process. ***Binding*** of a DBP analog produced by the mutant gene to the target sequence gives the cell a selective advantage; alternatively, the expression of the screenable gene is blocked. The method was used to modify the phage .lambda. Cro repressor to enable it to ***bind*** to an ***HIV*** -1 sequence. The selection system comprised a selectable gene, aadA (which confers

spectinomycin resistance), and a screenable gene, tet. The operon contg. the selectable gene consisted of the aadA gene with its natural promoter and occluding promoter Pcon followed by the target sequence. Upon ***binding*** of a DBP to the target sequence, expression from Pcon is inhibited and aadA is expressed. Tet gene expression was driven by Pneo.

L9 ANSWER 38 OF 77 CA COPYRIGHT 1999 ACS
AN 119:64797 CA
TI Mechanism of ***HIV*** -1 reverse transcriptase. Termination of processive synthesis on a natural DNA template is influenced by the sequence of the template-primer stem
AU Abbotts, John; Bebenek, Katarzyna; Kunkel, Thomas A.; Wilson, Samuel H.
CS Lab. Biochem., Natl. Cancer Inst., Bethesda, MD, 20892, USA
SO J. Biol. Chem. (1993), 268(14), 10312-23
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
AB During processive DNA synthesis in vitro, the human immunodeficiency virus, type 1 (***HIV*** -1) reverse transcriptase encounters template nucleotide positions at which continued synthesis is difficult. At these positions, the enzyme has a relatively high probability of dissocg. from the template, and product mols. of corresponding length accumulate as the incubation proceeds. These positions, which are known as termination sites, could be assocd. with template secondary structures in some cases, but many termination sites appear to be template sequence-related rather than secondary structure-related. Mechanisms producing these blocks in processive DNA synthesis are not well understood. In this study, to examine further the effects of template sequence on termination, the authors engineered selected single-base changes in the M13mp2 template, and found that such changes can influence termination. Several general trends emerged from the study. First, strong termination sites rarely correspond to dATP as the "incoming" substrate opposite template T. Second, the sequence of the template-primer stem is more important for termination than the sequence of the single-stranded template ahead of the primer. Thus, the authors note the phenomenon of action at a distance: changing sequence at one nucleotide position in the template-primer stem alters termination at other positions, a few nucleotides distant at the primer 3' end. A and C as template bases in the template-primer stem have opposite effects. A is the strongest terminator residue, and C is the weakest terminator residue, followed by G. Since termination sites are produced by reverse transcriptase dissocn. from the template-primer, the results suggest that the ***HIV*** -1 reverse transcriptase has properties reminiscent of a sequence-specific ***double*** - ***stranded*** DNA ***binding*** protein in that its ***binding*** mechanism can distinguish both base residues and positions in the

double - ***stranded*** DNA template-primer stem.

L9 ANSWER 39 OF 77 CA COPYRIGHT 1999 ACS
AN 119:63062 CA
TI Oligonucleotides useful as decoys for proteins which selectively ***bind*** to defined DNA sequences
IN Chu, Barbara Chen Fei; Orgel, Leslie
PA Salk Institute for Biological Studies, USA
SO PCT Int. Appl., 41 pp.
CODEN: PIXXD2
PI WO 9218522 A1 19921029
DS W: CA, JP, US
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE
AI WO 92-US3205 19920417
PRAI US 91-687337 19910418
DT Patent
LA English
AB ***Double*** - ***stranded*** oligonucleotides contg. a transcription control recognition sequence, the two strands of which are covalently connected by one nucleotide sequence to form a hairpin structure, or connected by two nucleotide sequences to form a circular structure, are claimed. The oligonucleotide may contain modified nucleotides, or may be modified so as to be capable of forming covalent bonds with the protein to which it ***binds*** . These oligonucleotides may be used to therapeutically modulate transcription. A closed, circular, self-complementary oligodeoxynucleotide structure contg. the recognition site for CRE-BP was prepd. This structure bound more efficiently to the protein than did a ***double*** - ***stranded*** DNA contg. the ***binding*** site, a hairpin structure contg. the ***binding*** site, or a circular, self-complementary structure which was not closed. The structure was covalently attached to CRE-BP by treatment with trans-Pt diammine dichloride. There was no discernible degrdn. of this complex after 24 h at 37.degree. in serum.

L9 ANSWER 40 OF 77 CA COPYRIGHT 1999 ACS
AN 119:43744 CA
TI Relatedness of an RNA- ***binding*** motif in human immunodeficiency virus type 1 TAR RNA- ***binding*** protein TRBP to human P1/dsI kinase and Drosophila staufen
AU Gatignol, Anne; Buckler, Charles; Jeang, Kuan Teh
CS Lab. Mol. Microbiol., Natl. Inst. Allergy Infect. Dis., Bethesda, MD, 20892, USA
SO Mol. Cell. Biol. (1993), 13(4), 2193-202
CODEN: MCEBD4; ISSN: 0270-7306
DT Journal

LA English
AB TRBP is a human cellular protein that ***binds*** the human immunodeficiency virus type 1 TAR RNA. Here it is shown that the intact presence of amino acids 247 to 267 in TRBP correlates with its ability to ***bind*** RNA. This region contains a lysine- and arginine-rich motif, KKLAKRNAAAKMLLRHTVPLDAR. A 24-amino-acid synthetic peptide (TR1) of this sequence bound TAR RNA with affinities similar to that of the entire TRBP, thus suggesting that this short motif contains a sufficient RNA-***binding*** activity. Using RNA probe-shift anal. it was detd. that TR1 does not ***bind*** all ***double*** - ***stranded*** RNAs but prefers TAR and other ***double*** - ***stranded*** RNAs with G+C-rich characteristics. Immunopptn. of TRBP from human immunodeficiency virus type 1-infected T lymphocytes recovered TAR RNA. This is consistent with a TRBP-TAR ribonucleoprotein during viral infection. Computer alignment revealed that TR1 is highly homologous to the RNA-***binding*** domain of human P1/dsI protein kinase and two regions within Drosophila Staufen. It is suggested that these proteins are related by virtue of sharing a common RNA- ***binding*** moiety.

L9 ANSWER 41 OF 77 CA COPYRIGHT 1999 ACS
AN 119:40338 CA
TI Mode of action of the anti-AIDS compound poly(I).cntdot.poly(C12U) (ampligen): Activator of 2',5'-oligoadenylate synthetase and ***double*** - ***stranded*** RNA-dependent kinase
AU Ushijima, Hiroshi; Rytik, Peter G.; Schaecke, Heike; Scheffer, Ute; Mueller, Werner E. G.; Schroeder, Heinz C.
CS Div. AIDS Virus, Natl. Inst. Health, Musashimurayama, 208, Japan
SO J. Interferon Res. (1993), 13(2), 161-71
CODEN: JIREDJ; ISSN: 0197-8357
DT Journal
LA English
AB The mismatched ***double*** - ***stranded*** RNA (dsRNA), poly(I).cntdot.poly(C12U), also termed Ampligen, exhibits a strong antiviral and cytoprotective effect on cells (human T-lymphoblastoid CEM cells and human T-cell line H9) infected with the human immunodeficiency virus type 1 (***HIV*** -1). Untreated H9 cells infected with ***HIV*** -1 start to release the virus 3 days post-infection, while in the presence of 40 .mu.g/mL (80 .mu.g/mL) of poly(I).cntdot.poly(C12U) the onset of virus prodn. and release is retarded and does not occur before day 5 (day 6). We demonstrate that poly(I).cntdot.poly(C12U) markedly extends the duration of the transient increase of 2',5'-oligoadenylate (2-5A) synthetase mRNA level and activity preceding virus prodn. after infection of cells with ***HIV*** -1. Treatment of HeLa cells with poly(I).cntdot.poly(C12U) was found to cause a significant increase in total (activated plus latent) 2-5A synthetase activity; no evidence was

obtained that the level of latent (nonactivated) 2-5A synthetase is changed in cells treated with dsRNA plus interferon (IFN). Poly(I).cntdot.polyC12U) is able to ***bind*** and to activate 2-5A synthetase(s) from HeLa cell exts. Addn. of poly(I).cntdot.poly(C12U) to HeLa cell exts. results in prodn. of longer 2-5A oligomers (.gtoreq.3 adenylate residues), which are better activators of RNase L. Both free and immobilized poly(I).cntdot.poly(C12U) also ***bind*** to the dsRNA-dependent protein kinase (p68 kinase), resulting in autophosphorylation of the enzyme. Activation of the kinase by the free RNA occurs within a limited concn. range (10-7 to 10-6 g/mL). Addn. of ***HIV*** -1 Tat protein does not affect ***binding*** and activation of p68 kinase to poly(I).cntdot.poly(C12U)-cellulose but strongly reduces the ***binding*** of the kinase to immobilized TAR RNA of ***HIV*** -1. We conclude that poly(I).cntdot.poly(C12U) may antagonize Tat-mediated down-regulation of dsRNA-dependent enzymes.

L9 ANSWER 42 OF 77 CA COPYRIGHT 1999 ACS
AN 119:26489 CA
TI Inhibition of the dsRNA-dependent protein kinase by a peptide derived from the human immunodeficiency virus type 1 Tat protein
AU Judware, Raymond; Li, Jinhe; Petryshyn, Raymond
CS Health Sci. Cent., State Univ. New York, Syracuse, NY, 13210, USA
SO J. Interferon Res. (1993), 13(2), 153-60
CODEN: JIREDJ; ISSN: 0197-8357
DT Journal
LA English
AB The human immunodeficiency virus (***HIV***) is the etiol. agent leading to the development of AIDS. Interferons (IFNs) are known for eliciting antiviral responses from cells, and studies have indicated that infection with ***HIV*** induces the prodn. of IFN. Previous studies have shown that the trans-acting response element (TAR) sequence of ***HIV*** -1 mRNA can activate the IFN-induced ***double*** - ***stranded*** (ds) RNA-dependent protein kinase (DAI). DAI, when activated, is a potent inhibitor of protein synthesis and has been implicated in mediating part of IFN's antiviral activity. Here is reported that a synthetic peptide contg. the basic region of ***HIV*** Tat protein is effective in preventing the activation of DAI. Evidence is presented that indicates that the Tat peptide exerts its effect by ***binding*** to the TAR RNA sequence and thus preventing this RNA from ***binding*** to and activating DAI. It appears that in addn. to its role in trans-activation, the Tat protein may also function to overcome the antiviral activity of IFN by regulating DAI activity. Thus, inhibition of DAI by the Tat protein early in the life cycle of ***HIV*** may provide a mechanism by which the virus can escape a translational block imposed by the kinase.

L9 ANSWER 43 OF 77 CA COPYRIGHT 1999 ACS
AN 118:251237 CA
TI ***Double*** - ***stranded*** strong-stop DNA and the second template switch in human immunodeficiency virus (***HIV***) DNA synthesis
AU Li, Peng; Stephenson, Alice J.; Kuiper, Lara J.; Burrell, Christopher J.
CS Natl. Cent. HIV Virol. Res., Inst. Med. Vet. Sci., Adelaide, 5000, Australia
SO Virology (1993), 194(1), 82-8
CODEN: VIRLAX; ISSN: 0042-6822
DT Journal
LA English
AB Synthesis of unintegrated retroviral DNA via reverse transcription is thought to involve 2 sep. template switches. Minus-strand strong-stop DNA, a major product of in vitro reactions using detergent-treated virions, is synthesized prior to the 1st template switch. Plus-strand strong-stop DNA, which is found base-paired to near full-length minus-strand DNA in infected cells, is believed to be synthesized before the 2nd template switch. Using a synchronized, 1-step ***HIV*** infection model, a novel ***double*** - ***stranded*** strong-stop ***HIV*** DNA with a discrete length of .apprx.650 base pairs commencing at or near the left hand end of the right-hand U3 region of the ***HIV*** long terminal repeat was detected in acutely infected cells. The plus-strand of this ***double*** - ***stranded*** strong-stop DNA possesses the primer ***binding*** site sequence and appears to be synthesized prior to the completion of the synthesis of its complementary minus-strand. In contrast, the minus-strand of the ***double*** - ***stranded*** strong-stop DNA lacks the primer ***binding*** site sequence after RNase H digestion. Apparently, a transient free plus-strand strong-stop DNA is released from its template by displacement synthesis and subsequently used as template for the synthesis of its complementary minus-strand. The proposed transient free plus-strand strong-stop DNA may also mediate the 2nd template switch.

L9 ANSWER 44 OF 77 CA COPYRIGHT 1999 ACS
AN 118:249284 CA
TI Mixed ligand complexes for use as luminescence labels for detection and photochemical cleavage of DNA
IN Barton, Jacqueline K.
PA Columbia University, USA
SO U.S., 87 pp. Cont.-in-part of U.S. 5,112,974.
CODEN: USXXAM
PI US 5157032 A 19921020
AI US 90-539930 19900618

PRAI US 85-693023 19850118
US 86-905295 19860908
US 88-268247 19881107
DT Patent
LA English
OS MARPAT 118:249284
AB Complexes M(R1)(R2)(R3) (M = transition metal, R1, R2, R3= ethylenediamine, bipyridine, phenanthroline, diazafluorene-9-one, phenanthrenequinonediimine, dipyridophenazine, or their substituted derivs.) are used as luminescence probes for detection of ***double*** - ***stranded*** DNA. These complexes show modified luminescence upon incorporation into a ***double*** - ***stranded*** DNA. These complexes can also be used to photochem. cleave DNA at a specific single-strand site and are useful as an anal. reagent and in the treatment of viral infections (e.g. with ***HIV***). 9,10-Phenanthrenequinone bis((-trimethylsilyl)-imine) 1.025 g, synthesized from 9,10-phenanthrenequinone and Na bis(trimethylsilyl)amide, in benzene 75 mL was mixed with a suspension of Ru(DMSO)4Cl2 0.355 g in an EtOH/benzene mixt. and incubated at 65.degree. until Ru(phi)3Cl2 formed; the product was collected by pptn. with di-Et ether. A series of analogs were also prepd. and their ***binding*** to DNA detd. by equil. dialysis; the spectroscopic properties of the complexes with DNA were also detd. ***Binding*** of the complexes to DNA was shown to be by intercalation. Photochem. cleavage of DNA with these complexes was base neutral and very clean. The use of these reagents in the killing of ***HIV*** -infected T4 lymphocytes.

L9 ANSWER 45 OF 77 CA COPYRIGHT 1999 ACS
AN 118:226757 CA
TI In vitro selection of fast-hybridizing and effective antisense RNAs directed against the human immunodeficiency virus type 1
AU Rittner, Karola; Burmester, Christoph; Sczakiel, Georg
CS Forschungsschwerpunkt Angew. Tumorvirol., Dtsch. Krebsforschungszent., Heidelberg, D-6900, Germany
SO Nucleic Acids Res. (1993), 21(6), 1381-7
CODEN: NARHAD; ISSN: 0305-1048
DT Journal
LA English
AB The rate of ***double*** ***strand*** formation between prokaryotic antisense RNA and complementary RNA in vitro is known to correlate with the effectiveness of antisense RNA in vivo. In this work, an in vitro assay for detg. the hybridization rates of a large no. of antisense RNA species was developed. A set of ***HIV*** -1-directed antisense RNAs with the same 5'-end but successively shortened 3'-ends was produced by alk. hydrolysis of a 150 nt ***HIV*** -1-directed antisense

transcript. This mixt. was used to det. hybridization rates for individual chain lengths with a complementary ***HIV*** -1-derived RNA in vitro. The second order ***binding*** rate consts. of individual antisense RNA species differed by more than a factor of 100, although in some cases, slow-hybridizing and fast-hybridizing antisense RNAs differed by only two or three 3'-terminally-located nucleotides. The results indicated that there was not a trivial dependence of ***binding*** rates on the chain length of antisense RNAs. Further, the ***binding*** rate consts. detd. in vitro for individual antisense RNA species correlated with the extent of inhibition of ***HIV*** -1 replication in vivo.

L9 ANSWER 46 OF 77 CA COPYRIGHT 1999 ACS
AN 118:206711 CA
TI DNA elements target of transcriptional factors are not restricted to long terminal repeat of human immunodeficiency virus
AU Feriotto, Giordana; Volinia, Stefano; Giacomini, Patrizio; Gambari, Roberto
CS Ist. Chim. Biol., Univ. Ferrara, Ferrara, Italy
SO Anticancer Res. (1992), 12(1), 65-71
CODEN: ANTRD4; ISSN: 0250-7005
DT Journal
LA English
AB It is shown that signals for transcriptional factors are not restricted to the ***HIV*** -1 LTR, but are present throughout the ***HIV*** -1 genome. Furthermore, a sequence, AGAACAGATG, was identified that is highly homologous to the X-box of class II MHC genes and located within the tat-IVS/env region of ***HIV*** -1. ***Double*** ***stranded*** oligonucleotides mimicking the ***HIV*** -1 region contg. AGAACAGATG were synthesized and band shift expts. were performed demonstrating that this ***HIV*** -1 genomic region ***binds*** nuclear proteins. The ***binding*** of nuclear factors to this tat-IVS/env ***HIV*** -1 sequence is competed for, in the band-shift assay, by the highly homologous X-box of the promoter of the human HLA-DR.alpha. gene. The presence in the ***HIV*** -1 genome of DNA sequences homologous or identical to regulatory sequences of cellular genes represents a potential mechanism of predation of DNA elements recognized by DNA ***binding*** proteins.

L9 ANSWER 47 OF 77 CA COPYRIGHT 1999 ACS
AN 118:186195 CA
TI Retroviral nucleocapsid proteins possess potent nucleic acid strand renaturation activity
AU Dib-Hajj, Fadia; Khan, Raza; Giedroc, David P.
CS Dep. Biochem. Biophys., Texas A and M Univ., College Station, TX,

77843-2128, USA
SO Protein Sci. (1993), 2(2), 231-43
CODEN: PRCIEI; ISSN: 0961-8368
DT Journal
LA English
AB The nucleocapsid protein (NC) is the major genomic RNA- ***binding*** protein that plays integral roles in the structure and replication of all animal retroviruses. Select biochem. properties of recombinant Mason-Pfizer monkey virus (MPMV) and ***HIV*** -1 NCs are compared. Two types of satd. Zn2NC-polynucleotide complexes can be formed under conditions of low [NaCl] that differ in apparent site-size (n = 8 vs. n = 14). The formation of one or the other complex appears dependent on the molar ratio of NC to RNA nucleotide, with the putative low site-size mode apparently predominating under conditions of protein excess. Both MPMV and ***HIV*** -1 NCs kinetically facilitate the renaturation of two cDNA strands, suggesting that this is a general property of retroviral NCs. NC proteins increase the second-order rate const. for renaturation of a 149-bp DNA fragment by more than four orders of magnitude over that obtained in the absence of protein at 37.degree.C. The protein-assisted rate is 100-200-fold faster than that obtained at 68.degree.C, 1 M NaCl, soln. conditions considered to be optimal for strand renaturation. Provided that sufficient NC is present to coat all strands, the presence of 400-1,000-fold excess nonhomologous DNA does not greatly affect the reaction rate. The ***HIV*** -1 NC-mediated renaturation reaction functions stoichiometrically, requiring a satd. strand of DNA nucleotide:NC ratio of about 7-8, rather than 14. Under conditions of less protein, the rate acceleration is not realized. The finding of significant nucleic acid strand renaturation activity may have important implications for various events of reverse transcription particularly in initiation and cDNA strand transfer.

L9 ANSWER 48 OF 77 CA COPYRIGHT 1999 ACS
AN 118:163562 CA
TI Conformational and nucleic acid ***binding*** studies on the synthetic nucleocapsid protein of ***HIV*** -1
AU Surovoy, Andrej; Dannull, Jens; Moelling, Karin; Jung, Gunther
CS Shemyakin Inst. Bioorg. Chem., Moscow, Russia
SO J. Mol. Biol. (1993), 229(1), 94-104
CODEN: JMOBAK; ISSN: 0022-2836
DT Journal
LA English
AB A 55 residue peptide corresponding to the nucleocapsid protein of ***HIV*** -1 (NCp7) contg. two zinc ***binding*** domains as well as three truncated peptides were synthesized by Fmoc-based solid phase synthesis using the fragment condensation approach. CD data support a

conformational model in trifluoroethanol/buffer soln. consisting of two helical segments at the chain ends with two Zn-modules in the center of the mol. CD titrn. expts. show that the synthetic protein ***binds*** two equiv. of Zn2+ stoichiometrically, and the Zn2+ induced conformational changes are completely reversible by addn. of EDTA. NCp7 and its S-acetamidomethylated analog (NCp7-Acm), devoid of the zinc co-ordination centers, exhibit preferential ***binding*** to RNA with a Kd =.apprx.10-9 M irresp. of the cysteine modification as detd. by filter ***binding*** assays. The ***binding*** affinity of the NCp7 protein to single-stranded DNA is lower than to RNA. ***Binding*** to ***double*** - ***stranded*** DNA is lower than to ssDNA. The NCp7-Acm protein exhibits reduced single-stranded DNA ***binding*** affinity compared to the unmodified protein. Nucleic acid ***binding*** analyses with the fragments of NCp& protein suggest that tow basic amino acid stretches are involved in RNA ***binding*** of the NCp7.

L9 ANSWER 49 OF 77 CA COPYRIGHT 1999 ACS
AN 118:119791 CA
TI Characterization of human immunodeficiency virus type 1 integrase expressed in Escherichia coli and analysis of variants with amino-terminal mutations
AU Vincent, Karen A.; Ellison, Viola; Chow, Samson A.; Brown, Patrick O.
CS Sch. Med., Stanford Univ., Stanford, CA, 94305, USA
SO J. Virol. (1993), 67(1), 425-37
CODEN: JOVIAM; ISSN: 0022-538X
DT Journal
LA English
AB Replication of a retroviral genome depends upon integration of the viral DNA into a chromosome of the host cell. The integration reaction is mediated by integrase, a viral enzyme. Human immunodeficiency virus type 1 integrase was expressed in E. coli and purified to near homogeneity. Optimum conditions for the integration and 3'-end-processing activities of integrase were characterized by using an in vitro assay with short, ***double*** - ***stranded*** oligonucleotide substrates. Mutants contg. amino acid substitutions within the HHCC region, defined by phylogenetically conserved of histidine and cysteine residues near the N terminus, were constructed and characterized by using three assays: 3'-end processing, integration, and the reverse of the integration reaction (or disintegration). Mutations in the conserved histidine and cysteine residues abolished both integration and processing activities. Weak activity in both assays were retained by two other mutants contg. substitutions for less highly conserved amino acids in this region. All mutants retained activity in the disintegration assay, implying that the active site for DNA cleavage-ligation is not located in this domain and that the HHCC region is not the sole DNA- ***binding*** domain in the

protein. However, the preferential impairment of processing and integration rather than disintegration by mutations in the HHCC region is consistent with a role for this domain in recognizing features of the viral DNA. This hypothesis is supported by the results of disintegration assays performed with altered substrates. The results support a model involving sep. viral and target DNA- ***binding*** sites on integrase.

L9 ANSWER 50 OF 77 CA COPYRIGHT 1999 ACS
AN 118:96878 CA
TI RNase D, a reported new activity associated with ***HIV*** -1 reverse transcriptase, displays the same cleavage specificity as Escherichia coli RNase III
AU Hostomsky, Zdenek; Hudson, Geoffrey O.; Rahmati, Soheil; Hostomska, Zuzana
CS Agouron Pharm., Inc., San Diego, CA, 92121, USA
SO Nucleic Acids Res. (1992), 20(21), 5819-24
CODEN: NARHAD; ISSN: 0305-1048
DT Journal
LA English
AB RNase D was recently reported as a new enzymic activity assocd. with ***HIV*** -1 reverse transcriptase (RT), cleaving RNA at 2 positions within the ***double*** - ***stranded*** region of the tRNA primer-viral RNA template complex. This would make RNase D a 4th distinct activity of ***HIV*** -1 RT, in addn. to RNA- and DNA-dependent DNA polymerases and RNase H. Using a specific substrate contg. tRNALys,3 hybridized to the primer ***binding*** site, it was possible to detect the reported RNase D activity in the prepns. of recombinant ***HIV*** -1 RT. This activity was also present in several active-site mutants of RT, suggesting that it is independent of the RNase H and polymerase functionalities of RT. Furthermore, it was found that the cleavage specificity of RNase D was the same as that of RNase III isolated from E. coli. A likely explanation of these results, that the obsd. RNase D activity is attributable to traces of RNase III contamination, was further strengthened by the finding that the recombinant prepns. of ***HIV*** -1 RT can specifically cleave a phage T7-derived ***double*** - ***stranded*** RNA processing signal, which has been used as a model substrate for detection of E. coli RNase III. Moreover, RT purified from an RNase III-strain of E. coli displayed no cleavage of the tRNA primer-RNA template complex.

L9 ANSWER 51 OF 77 CA COPYRIGHT 1999 ACS
AN 118:17101 CA
TI Incorporation of single-stranded DNA ***binding*** protein early in polymerase chain reaction product sequencing reactions prevents enzyme pausing
AU Ball, Jonathan K.; Desselberger, Ulrich

CS Re. Virus Lab., East Birmingham Hosp., Birmingham, B9 5ST, UK
SO Anal. Biochem. (1992), 207(2), 349-51
CODEN: ANBCA2; ISSN: 0003-2697
DT Journal
LA English
AB Premature pausing of DNA polymerase in the DNA sequencing reaction due to secondary structure in the template or reannealing of ***double*** - ***stranded*** PCR products was prevented by addn. of single-stranded DNA- ***binding*** protein to the sequencing reaction. Proteins g32 and SSBP were added prior to heat denaturation of the template for the DNA sequencing reaction during direct sequencing after PCR amplification of the third variable region of gene env of ***HIV*** -1, a region where other methods of preventing enzyme pausing had failed. The proteins were destroyed prior to electrophoresis by using a stop soln. contg. guanidinium isothiocyanate. Addn. of SSBP resulted in enzyme read-through of the region prone to premature termination due to enzyme pausing; g32 had no effect on enzyme pausing.

L9 ANSWER 52 OF 77 CA COPYRIGHT 1999 ACS
AN 117:206336 CA
TI Transcription-based nucleic acid amplification system by two-enzyme, self-sustained sequence replication
IN Fahy, Eoin David; Kwoh, Deborah Yantis; Gingeras, Thomas Raymond; Guatelli, John Christopher; Whitfield, Kristina Marie
PA Siska Diagnostics, Inc., USA
SO PCT Int. Appl., 95 pp.
CODEN: PIXXD2
PI WO 9208800 A1 19920529
DS W: AT, AU, BB, BG, BR, CA, CH, CS, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MC, MG, MN, MW, NL, NO, PL, RO, SD, SE, SU
RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IT, LU, ML, MR, NL, SE, SN, TD, TG
AI WO 91-US8488 19911113
PRAI US 90-612688 19901113
DT Patent
LA English
OS MARPAT 117:206336
AB A transcription-based nucleic acid amplification system (TAS) uses RNA-dependent DNA polymerase, DNA-dependent DNA polymerase, RNase H, and DNA-dependent RNA polymerase activities for detecting a target RNA is described. The method transcribes an RNA to cDNA that is converted to the ***double*** - ***stranded*** form that is then transcribed to give an RNA for another round of conversion to the cDNA. The system also requires primers that contain a promoter sense sequence and does not require thermal denaturation between each round of amplifications. RNase H, e.g.

from Escherichia coli, may be added to improve efficiency. The system is optimized by addn. to the reaction medium of >1 of C1-10 alc., a sugar alc., a polyethylene glycol, a sugar, and a sulfoxide. Detection of the cystic fibrosis-assocd. gene is demonstrated. The RNA was amplified using reverse transcriptase (avian myeloblastosis virus) for 1 min, then incubated with reverse transcriptase, T7 RNA polymerase, and RNAase H. The amplified nucleic acid was detected by ***binding*** to immobilized oligonucleotides.

L9 ANSWER 53 OF 77 CA COPYRIGHT 1999 ACS
AN 117:206219 CA
TI RNA ***binding*** assays for Tat-derived peptides: implications for specificity
AU Weeks, Kevin M.; Crothers, Donald M.
CS Dep. Chem., Yale Univ., New Haven, CT, 06511, USA
SO Biochemistry (1992), 31(42), 10281-7
CODEN: BICHAW; ISSN: 0006-2960
DT Journal
LA English
OS CJACS
AB RNA recognition by the ***HIV*** Tat protein is mediated in part by an arginine- and lysine-rich basic subdomain implicated as a signature element in proteins that ***bind*** RNA. Relative RNA ***binding*** affinities for a 14-residue peptide derived from Tat that spans the basic region are detd. using a competition protocol. ***Binding*** specificity is compared with complexation by a 38-residue model for the RNA- ***binding*** domain of Tat using the same approach. ***Binding*** strength for the minimal (14 residue) peptide is correlated with that for the longer peptide: both peptides recognize a short, bulged duplex. However, the shorter peptide dissocs. more rapidly from the wild-type site and discriminates less well between nonspecific (***double*** - ***stranded*** RNA) and specific sites. Relative dissocn. consts. for the 38-residue peptide detd. from direct partition and competition assays differ; the former assay consistently predicts stronger discrimination against RNAs with mutations in the stems flanking the bulge. Differences between the 2 assays are reconciled in terms of contributions from labile ***binding*** which is unstable to native gel electrophoresis. Kinetic stability may constitute a major specificity determinant for basic subdomain-mediated recognition of RNA.

L9 ANSWER 54 OF 77 CA COPYRIGHT 1999 ACS
AN 117:127156 CA
TI Interaction of tRNALys with the p66/p66 form of ***HIV*** -1 reverse transcriptase stimulates DNA polymerase and ribonuclease H activities
AU Andreola, Marie Line; Nevinskii, G. A.; Barr, Philip J.; Sarih-Cottin,

Leila; Bordier, Bruno; Fournier, Michel; Litvak, Simon; Tarrago-Litvak, Laura

CS Inst. Biochim. Cell. Neurochim., Cent. Natl. Rech. Sci., Bordeaux, 33077, Fr.

SO J. Biol. Chem. (1992), 267(27), 19356-62
CODEN: JBCHA3; ISSN: 0021-9258

DT Journal

LA English

AB The precursor homodimeric p66/p66 form of human immunodeficiency virus type-1 reverse transcriptase (***HIV*** -1 RT) possesses the DNA polymerase and RNase H activities involved in the synthesis of the ***double*** - ***stranded*** provirus DNA. Reverse transcription is initiated from rRNALys in the case of ***HIV*** -1. The present study confirmed that interactions between ***HIV*** -1 RT and tRNALys induce protein conformational changes and demonstrated that these interactions stimulate the enzymic activities assocd. with the p66 subunit. Thus, the p66/p66 form of the enzyme is strongly stimulated in both DNA polymerase and RNase H activities. Preincubation of the enzyme with tRNA is an obligatory step to obtain the stimulatory effect. The affinity of template, primer, or substrate for RT p66/p66 did not change when the enzyme was preincubated with tRNALys at stimulatory concns.; the interaction of tRNA with p66/p66 has an effect only on the maximal rate of polymn. It is further shown that the RNase H domain of RT is much more accessible to protease attack than the DNA polymerase active site.

L9 ANSWER 55 OF 77 CA COPYRIGHT 1999 ACS

AN 117:107193 CA

TI Structure of ***HIV*** -1 reverse transcriptase/DNA complex at 7 .ANG. resolution showing active site locations

AU Arnold, Edward; Jacobo-Molina, Alfredo; Nanni, Raymond G.; Williams, Roger L.; Lu, Xiaode; Ding, Jianping; Clark, Arthur D., Jr.; Zhang, Anqiang; Ferris, Andrea L.; et al.

CS Cent. Adv. Biotechnol. Med., Piscataway, NJ, 08854-5638, USA

SO Nature (London) (1992), 357(6373), 85-9
CODEN: NATUAS; ISSN: 0028-0836

DT Journal

LA English

AB The structure at 7-.ANG. resoln. is reported of a ternary complex of the ***HIV*** -1 reverse transcriptase heterodimer, a monoclonal antibody Fab fragment, and a duplex DNA template-primer. ***Double*** - ***stranded*** DNA ***binds*** in a groove on the surface of the enzyme. The electron d. near one end of the DNA matches well with the known structure of the ***HIV*** -1 reverse transcriptase RNase H domain. At the opposite end of the DNA, a mercurated deriv. of UTP has been localized by the difference Fourier method, allowing tentative

identification of the polymerase nucleoside triphosphate ***binding*** site. The structure of the reverse transcriptase/Fab complex was also reported in the absence of template-primer to compare the bound and free forms of the enzyme. The presence of DNA correlates with movement of protein electron d. in the vicinity of the putative template-primer ***binding*** groove.

L9 ANSWER 56 OF 77 CA COPYRIGHT 1999 ACS
AN 117:65042 CA
TI Nucleic acid interactive properties of a peptide corresponding to the N-terminal zinc finger domain of ***HIV*** -1 nucleocapsid protein
AU Delahunty, Martha D.; South, Terri L.; Summers, Michael F.; Karpel, Richard L.
CS Dep. Chem. Biochem., Univ. Maryland, Baltimore, MD, 21228, USA
SO Biochemistry (1992), 31(28), 6461-9
CODEN: BICHAW; ISSN: 0006-2960
DT Journal
LA English
OS CJACS
AB An 18-residue peptide (NC-F1) with an amino acid sequence corresponding to the first zinc finger of human immunodeficiency virus-1 nucleocapsid protein has been shown to ***bind*** to nucleic acids by fluorescence and NMR methods. Previously, this peptide was shown to fold into a defined structure when bound to zinc. A fluorescent polynucleotide, poly(ethenoadenylic acid), was used to monitor ***binding*** of this peptide to nucleic acids. In the presence of zinc, the peptide had a smaller site size (1.75 nucleotide residues/peptide) than in the absence of the metal ion (2.75). The salt sensitivity of the interaction indicated that two ion pairs are involved in the assocn. of Zn2+(NC-F1) with polynucleotide, whereas one ion pair is found in the metal-free peptide-nucleic acid complex. Competition expts. with single-stranded DNA (ss DNA) in either the presence or absence of Zn2+ showed that the peptide bound to ss DNA. The peptide also bound to ***double*** - ***stranded*** DNA (ds DNA) with approx. the same affinity, indicating the absence of specificity for secondary structure. Using NMR methods, the ***binding*** of a synthetic oligonucleotide, d(TTTGGTTT), to Zn(NC-F1) was monitored. The hydrophobic residues F2 and I10, which are on the surface of the peptide and have been implicated in viral RNA recognition, interact with the oligomer. In accord with this observation, anal. of the salt dependence of the polynucleotide-peptide interaction indicates a nonelectrostatic component of about -6 kcal/mol, a value consistent with theor. ests. of stacking energies of phenylalanine with nucleic acid bases.

L9 ANSWER 57 OF 77 CA COPYRIGHT 1999 ACS

AN 116:230318 CA
TI Specific ***binding*** of arginine to TAR RNA
AU Tao, Jianshi; Frankel, Alan D.
CS Whitehead Inst. Biomed., Cambridge, MA, 02142, USA
SO Proc. Natl. Acad. Sci. U. S. A. (1992), 89(7), 2723-6
CODEN: PNASA6; ISSN: 0027-8424
DT Journal
LA English
AB A single arginine residue within the basic region of the human immunodeficiency virus Tat protein mediates specific ***binding*** of Tat peptides to a three-nucleotide bulge in TAR RNA. It has been proposed that arginine recognizes TAR by forming a network of hydrogen bonds with two structurally distinct phosphates, an interaction termed the arginine fork. Here it is shown that L-arginine blocks the Tat peptide/TAR interaction, whereas L-lysine and analogs of arginine that remove specific hydrogen bond donors do not. Expts. using an L-arginine affinity column demonstrate that arginine and the Tat peptides ***bind*** to the same site in TAR. Modification of two phosphates located at the junction of the ***double*** - ***stranded*** stem and bulge and modification of two adenine N7 groups in base-paired regions of TAR interfere with specific arginine ***binding*** . The results emphasize the importance of RNA structure in RNA-protein recognition and provide methods to identify arginine- ***binding*** sites in RNAs.

L9 ANSWER 58 OF 77 CA COPYRIGHT 1999 ACS
AN 116:209876 CA
TI Specific ***binding*** of a basic peptide from ***HIV*** -1 Rev
AU Kjems, Jorgen; Calnan, Barbara J.; Frankel, Alan D.; Sharp, Philip A.
CS Cent. Cancer Res., Massachusetts Inst. Technol, Cambridge, MA, 02139-4307, USA
SO EMBO J. (1992), 11(3), 1119-29
CODEN: EMJODG; ISSN: 0261-4189
DT Journal
LA English
AB Human immunodeficiency virus type I (***HIV*** -1) encodes a regulatory protein, Rev, which is required for cytoplasmic expression of incompletely spliced viral mRNA. Rev activity is mediated through specific ***binding*** to a cis-acting Rev responsive element (RRE) located within the env region of ***HIV*** -1. A monomer Rev ***binding*** site corresponding to 37 nucleotides of the RRE (IIB RNA) was studied by RNA footprinting, modification interference expts. and mutational anal. Surprisingly, a 17 amino acid peptide, corresponding to the basic domain of Rev, ***binds*** specifically to this site at essentially identical nucleotides and probably induces addnl. base pairing. The Rev protein and related peptide interact primarily with 2 sets of nucleotides located at

the junction of single and ***double*** ***stranded*** regions, and at an addnl. site located within a helix. This suggests that the domains of proteins responsible for specific RNA ***binding*** can be remarkably small and that the interaction between RNA and protein can probably induce structure in both constituents.

L9 ANSWER 59 OF 77 CA COPYRIGHT 1999 ACS
AN 116:101656 CA
TI ***Double*** - ***stranded*** RNA-dependent RNase activity associated with human immunodeficiency virus type 1 reverse transcriptase
AU Ben-Artzi, Hana; Zeelon, Elisha; Gorecki, Marian; Panet, Amos
CS BioTechnol. Gen. (Israel) Ltd., Rehovot, 76326, Israel
SO Proc. Natl. Acad. Sci. U. S. A. (1992), 89(3), 927-31
CODEN: PNASA6; ISSN: 0027-8424
DT Journal
LA English
AB Early events in the retroviral replication cycle include the conversion of viral genomic RNA into linear ***double*** - ***stranded*** DNA. This process is mediated by the reverse transcriptase (RT), a multifunctional enzyme that possesses RNA-dependent DNA polymerase, DNA-dependent DNA polymerase, and RNase H activities. In the course of studies of a recombinant RT of human immunodeficiency virus type 1 (***HIV*** -1), an addnl., unexpected activity of the enzyme was obsd. The purified RT catalyzed a specific cleavage in ***HIV*** -1 RNA hybridized to tRNALys, the primer for ***HIV*** -1 reverse transcription. The cleavage at the primer ***binding*** site (PBS) of ***HIV*** RNA was dependent on the ***double*** - ***stranded*** structure of the ***HIV*** RNA-tRNALys complex. This RNase activity appeared to be distinct from the RNase H activity of ***HIV*** -1 RT, as the substrate specificity and the products of the 2 activities were different. Moreover, Escherichia coli RNase H and avian myeloblastosis virus RT were unable to cleave the ***HIV*** RNA-tRNALys complex. This unusual activity was referred to as RNase D. Two lines of evidence indicated that the specific RNase D activity is an integral part of recombinant ***HIV*** RT. The specific RNase D activity comigrated with the other RT activities, DNA polymerase, and RNase H upon filtration on a Superose 6 gel column or chromatog. on a phosphocellulose column. Moreover, 3 recombinant ***HIV*** -1 RT prepns. expressed and purified in different labs. by various procedures exhibited RNase D activity. Sequence anal. indicated that RNase D activity cleaves the substrate ***HIV*** -1 RNA-tRNALys at 2 distinct sites within the PBS sequence 5'-UGGCGCCCGA .dwnarw. ACAG .dwnarw. GGAC-3'. The sequence specificity of RNase D activity suggested that it might be involved in 2 stages during the reverse transcription process: displacement of the PBS to enable copying of tRNALys sequences into plus-strand DNA or to facilitate the 2nd

template switch, which was postulated to occur at the PBS sequence.

L9 ANSWER 60 OF 77 CA COPYRIGHT 1999 ACS
AN 115:201334 CA
TI Triple-helix formation by .alpha. oligodeoxynucleotides and .alpha. oligodeoxynucleotide-intercalator conjugates
AU Sun, J. S.; Giovannangeli, C.; Francois, J. C.; Kurfurst, R.; Montenay-Garestier, T.; Asseline, U.; Saison-Behmoaras, T.; Thuong, N. T.; Helene, C.
CS Lab. Biophys., Mus. Natl. Histoire Nat., Paris, 75005, Fr.
SO Proc. Natl. Acad. Sci. U. S. A. (1991), 88(14), 6023-7
CODEN: PNASA6; ISSN: 0027-8424
DT Journal
LA English
AB Base-pair sequences in ***double*** - ***stranded*** DNA can be recognized by homopyrimidine oligonucleotides that ***bind*** to the major groove at homopurine.cntdot.homopyrimidine sequences thereby forming a local triple helix. To make oligodeoxynucleotides resistant to nucleases, the natural (.beta.) anomers of the nucleotide units were replaced by the synthetic (.alpha.) anomers. The 11-mer .alpha. oligodeoxynucleotide 5'-d(TCTCCTCCTTT)-3' ***binds*** to the major groove of DNA in an antiparallel orientation with respect to the homopurine strand, whereas a .beta. oligonucleotide adopts a parallel orientation. When an intercalating agent was attached to the 3' end of the .alpha. oligodeoxynucleotide, a strong stabilization of the triple helix was obsd. A 16-base-pair homopurine.cntdot.homopyrimidine sequence of human immunodeficiency virus proviral DNA was chosen as a target for a 16-mer homopyrimidine .alpha. oligodeoxynucleotide. A restriction enzyme that cleaves DNA at the junction of the homopurine.cntdot.homopyrimidine sequence was inhibited by triple-helix formation. The 16-mer .alpha. oligodeoxynucleotide substituted by an intercalating agent was .apprxeq.20 times more efficient than the unsubstituted oligomer. Nuclease-resistant .alpha. oligodeoxynucleotides offer addnl. possibilities to control gene expression at the DNA level.

L9 ANSWER 61 OF 77 CA COPYRIGHT 1999 ACS
AN 115:177792 CA
TI Arginine-mediated RNA recognition: the arginine fork
AU Calnan, Barbara J.; Tidor, Bruce; Biancalana, Sara; Hudson, Derek; Frankel, Alan D.
CS Whitehead Inst. Biomed. Res., Cambridge, MA, 02142, USA
SO Science (Washington, D. C., 1883-) (1991), 252(5009), 1167-71
CODEN: SCIEAS; ISSN: 0036-8075
DT Journal
LA English

AB Short peptides that contain the basic region of the ***HIV*** -1 Tat protein ***bind*** specifically to a bulged region in TAR RNA. A peptide that contained nine arginines (R9) also bound specifically to TAR, and a mutant Tat protein that contained R9 was fully active for transactivation. In contrast, a peptide that contained nine lysines (K9) bound TAR poorly and the corresponding protein gave only marginal activity. By starting with the K9 mutant and replacing lysine residues with arginines, a single arginine was identified that is required for specific ***binding*** and transactivation. Ethylation interference expts. suggest that this arginine contacts two adjacent phosphates at the RNA bulge. Model building suggests that the arginine .eta. N and the .epsilon. N can form specific networks of H bonds with adjacent pairs of phosphates and that these arrangements are likely to occur near RNA loops and bulges and not within ***double*** - ***stranded*** A-form RNA. Thus, arginine side chains may be commonly used to recognize specific RNA structures.

L9 ANSWER 62 OF 77 CA COPYRIGHT 1999 ACS
AN 115:152137 CA
TI Replication of the human immunodeficiency virus type 1 is inhibited by the adeno-associated virus rep gene
AU Rittner, Karola; Heilbronn, Regine; Kleinschmidt, Juergen A.; Oelze, Ingo; Sczakiel, Georg
CS Inst. Virusforsch., Deutsch. Krebsforschungszent., Heidelberg, D-6900, Fed. Rep. Ger.
SO Biochem. Soc. Trans. (1991), 19(4), 438S
CODEN: BCSTB5; ISSN: 0300-5127
DT Journal
LA English
AB Infectious proviral ***HIV*** -1 clone pNL4-3 was microinjected into human epitheloid SW480 cells. By co-cultivating human lymphoid MT-4 cells, ***HIV*** -1 initially produced in microinjected cells was amplified and could be measured. Effects of cointroduced adeno-assocd. virus (AAV) DNA on the first cycle of ***HIV*** -1 replication in microinjected cells were detd. by a com. ***HIV*** -1 antigen ELISA with cell free co-culture supernatants. Complete cloned AAV-2 DNA inhibited replication of ***HIV*** -1 by >95%. Further, mutational analyses of the AAV-2 genome showed that the AAV-2 encoded rep gene was necessary for this effect. Further, mutants of the rep p78 protein showed that an intact ATP ***binding*** site and portions between the start site of p78 rep and p52 rep were required for inhibition. A sequence comparison between the AAV-terminal repeat which is known to ***bind*** the rep protein and the ***HIV*** -1 long terminal repeat (LTR) revealed a 25-bp stretch with 72% sequence identity (AHH) which is located within the TAR region of ***HIV*** -1 (+30 to +54). A ***HIV*** -1

LTR-driven chloramphenicol acetyltransferase (CAT) gene together with cloned AAV-2 DNA and eukaryotic expression plasmids contg. the intact or mutated rep open reading frames, resp., were coinjected with SW480 cells. A significant and dose-dependent inhibition of CAT expression in the presence of an intact rep gene was shown. In the same expt. the rep-mediated inhibition of ***HIV*** -1 LTR-driven CAT expression was competed, i.e. the extent of inhibition was decreased when isolated ***HIV*** -1 DNA fragments or short ***double*** - ***stranded*** oligonucleotides contg. the AHH element were co-microinjected. Insertion of the AHH element from ***HIV*** -1 (AHHH) into a heterologous expression cassette, i.e. a HCMV-driven CAT gene, led to rep-dependent down regulation of CAT expression which was not obsd. with the parental HCMV CAT construct. This indicates that the AHHH element is necessary for the neg. effects of AAV-2 on ***HIV*** -1 functions.

L9 ANSWER 63 OF 77 CA COPYRIGHT 1999 ACS
AN 115:67085 CA
TI Nickel(II) porphyrin ***binding*** to anionic biopolymers investigated by resonance Raman and optical spectroscopy
AU Yue, K. T.; Lin, Mengfen; Gray, Thomas A.; Marzilli, Luigi G.
CS Dep. Chem., Emory Univ., Atlanta, GA, 30322, USA
SO Inorg. Chem. (1991), 30(16), 3214-22
CODEN: INOCAJ; ISSN: 0020-1669
DT Journal
LA English
OS CJACS
AB Metalloporphyrins possess activity against ***HIV*** -1, the virus responsible for AIDS. In addn., some porphyrins with cationic charge also have anticancer activity and are interesting probes of nucleic acid properties. Ni(II) derivs. of meso-tetrakis(4-N-methylpyridiniumyl)porphyrin, NiTMpyP(4), and meso-tetrakis(2-N-methylpyridiniumyl)porphyrin, NiTMpyP(2), are particularly useful for study. In an aq. soln., these nickel porphyrins exist in a 6-(diaqua) to 4-coordinate equil. mixt. The equil. position is dependent on the environment and on polymer ***binding*** . Resonance Raman spectroscopy is an esp. powerful means for assessing this equil. because well-defined bands exist for each form. The utility of the resonance Raman method is enhanced when it is used in conjunction with optical spectroscopy. Resonance Raman studies have previously focused on porphyrin ***binding*** to ***double*** - ***stranded*** DNA. The authors extended the application of the method to other types of polymers, including single-stranded DNA and RNA, polypeptides, and sulfated carbohydrates. Resonance Raman studies from the Nakamoto lab. have revealed that, except for [poly(dAdT)]2, a relatively unperturbed 4-coordinate form of NiTMpyP(4) dominated for duplex DNA bound forms at

all ratios (R) of porphyrin to polymer. This relatively unperturbed 4-coordinate form predominates with the newly investigated polymers also. Typically, 6-coordinate NiTMpyP(4) is not bound to polymers. However, at high R values, an unperturbed 6-coordinate form of NiTMpyP(4) is bound to poly(U), poly(dU), and dextran sulfate. The studies allow one to classify the various polymers based on their effectiveness in converting the 6-coordinate forms of NiTMpyP(4) and NiTMpyP(2) to the resp. 4-coordinate forms. Nakamoto reported that a unique, perturbed 4-coordinate species formed with [poly(dAdT)]2 at 0.2 M NaCl. The authors' [poly(dAdT)]2 studies were conducted at a relatively low salt concn. (0.01M NaCl). New CD and absorption spectroscopic studies under both conditions reveal that the low salt conditions promote the formation of a previously unrecognized spectroscopically distinct form of NiTMpyP(4) bound to [poly(dAdT)]2 at high R. The authors report evidence that this species may involve porphyrin-porphyrin stacking includes a hypochromic effect in the absorption spectrum and a conservative CD spectrum in the Soret region. At lower R values or at all R values at higher salt, this stacked species was not obsd. Instead, the spectra were similar, with a hyperchromic Soret band and a less conservative CD spectrum dominated by a pos. band at ca. 415 nm. Although the optical spectra were different, the broadening of the Raman spectral band at ca. 1097 cm-1, characteristic of the unique 4-coordinate species, was obsd. in both cases. The [poly(dAdT)]2 polymer is weakly stacked, with an unhindered minor groove. The weak stacking leads to flexibility. Apparently, flexibility is not sufficient to induce the formation of the unique 4-coordinate form of NiTMpyP(4), since the broadening of the ca. 1097-cm-1 band was not obsd. with either the single-stranded nucleic acids or the other polymers studied here. Formation of the unique species identified by resonance Raman spectroscopy may require interaction of the porphyrin with an unhindered minor groove in a duplex.

L9 ANSWER 64 OF 77 CA COPYRIGHT 1999 ACS
AN 114:243135 CA
TI Characterization of ***HIV*** -1 REV protein: ***binding*** stoichiometry and minimal RNA substrate
AU Cook, Kathleen Sue; Fisk, Gregory J.; Hauber, Joachim; Usman, Nassim; Daly, Thomas J.; Rusche, James R.
CS Repligen Corp., Cambridge, MA, 02139, USA
SO Nucleic Acids Res. (1991), 19(7), 1577-83
CODEN: NARHAD; ISSN: 0305-1048
DT Journal
LA English
AB The ***HIV*** -1 REV protein ***binds*** to the stem II region of the REV-responsive element (RNA). Studies to further define the RNA sequence and structure specifically bound by REV protein identify a

minimal RNA element of 40 nucleotides. Anal. of RNA fragments by gel retardation and filter ***binding*** suggest that a core element composed of one particular stem with flanking sequences capable of forming a second ***double*** ***stranded*** region is essential for specific recognition by REV protein. Stable REV-RNA complexes are formed in a stoichiometry of 1 REV:1 RNA. The minimal RNA element ***binds*** 1 REV mol. while the stem II sats. at 3 REV mols. per RNA. These results establish that REV recognizes a primary ***binding*** site within the REV responsive element and support the notion that the initial viral transcript ***binding***. event involves a monomeric REV protein.

L9 ANSWER 65 OF 77 CA COPYRIGHT 1999 ACS
AN 114:118339 CA
TI A key DNA-protein interaction determines the function of the 5'URR enhancer in human papillomavirus type 11
AU Auborn, K. J.; Steinberg, B. M.
CS Dep. Otolaryngol., Long Island Jew. Med. Cent., New Hyde Park, NY, 11042, USA
SO Virology (1991), 181(1), 132-8
CODEN: VIRLAX; ISSN: 0042-6822
DT Journal
LA English
AB The 5' end of the upstream regulatory region (URR) of human papillomavirus type 11 (***HPV*** -11) has enhancer function and ***binds*** cellular proteins. It was detd. that one particular motif, a CCNGTNAC pair, is both necessary and sufficient for enhancer activity. This enhancement of expression can be competed in vivo by concateneate ***double*** - ***stranded*** oligonucleotides, indicating that protein-DNA ***binding*** is a requisite for enhancer activity. A 41-kDa protein, present in all epithelial cells assayed, ***binds*** to this enhancer motif. The 5'URR fragment functions as an enhancer both in primary keratinocytes from a variety of body sites and in fibroblasts. It was concluded that tissue specificity is not a feature of this enhancer, and that the 41-kDa ***binding*** protein is ubiquitous. These data provide evidence that the 5'URR enhancer activity is dependent on only a few sequences and perhaps only one protein.

L9 ANSWER 66 OF 77 CA COPYRIGHT 1999 ACS
AN 114:95944 CA
TI The integrity of the stem structure of human immunodeficiency virus type 1 Tat-responsive sequence RNA is required for interaction with the interferon-induced 68,000-Mr protein kinase
AU Roy, Sophie; Agy, Michael; Hovanessian, Ara G.; Sonenberg, Nahum; Katze, Michael G.
CS McGill Cancer Cent., McGill Univ., Montreal, PQ, H3G 1Y6, Can.

SO J. Virol. (1991), 65(2), 632-40
CODEN: JOVIAM; ISSN: 0022-538X
DT Journal
LA English
AB A no. of eukaryotic viruses have devised strategies to minimize the deleterious effects on protein synthesis caused by activation of the interferon-induced, ***double*** - ***stranded*** -RNA-activated protein kinase, P68. In a recent report, the down regulation of the P68 protein kinase in cells infected by human immunodeficiency virus type 1 (***HIV*** -1) was described (Roy, S., et al., 1990). This report presents evidence that such a decrease in amts. of P68 could be essential for ***HIV*** -1 replication because of the presence of the Tat-responsive sequence (TAR sequence) present in the 5' untranslated region of ***HIV*** -1 mRNAs, which activates the P68 kinase. Poly(A)+ mRNAs prepd. from ***HIV*** -1-infected cells efficiently activated the protein kinase as did mRNAs from stably transformed cell lines constitutively expressing the TAR region. Furthermore, TAR-contg. RNAs complexed with purified P68 protein kinase in vitro by 2 independent assays and could be cross-linked to P68 kinase present in a HeLa cell ext. Expts. using a vitro-synthesized wild-type and mutant TAR RNAs revealed that both the efficient ***binding*** to and the activation of P68 kinase were dependent on the TAR RNA stem structure. The TAR-P68 complex could be competed out by a synthetic RNA that bound to and activated the protein kinase but not by a synthetic RNA that bound with low affinity and did not activate P68. The possible biol. consequences of a P68-TAR interaction that may include the switch from latent to active virus replication are discussed.

L9 ANSWER 67 OF 77 CA COPYRIGHT 1999 ACS
AN 114:38090 CA
TI Fragments of the ***HIV*** -1 Tat protein specifically ***bind*** TAR RNA
AU Weeks, Kevin M.; Ampe, Christophe; Schultz, Steve C.; Steitz, Thomas A.; Crothers, Donald M.
CS Dep. Chem., Yale Univ., New Haven, CT, USA
SO Science (Washington, D. C., 1883-) (1990), 249(4974), 1281-5
CODEN: SCIEAS; ISSN: 0036-8075
DT Journal
LA English
AB Proteolytically produced carboxyl-terminal fragments of the human immunodeficiency virus type-1 (***HIV*** -1) Tat protein that include a conserved region rich in arginine and lysine ***bind*** specifically to transactivation response RNA sequences (TAR). A chem. synthesized 14-residue peptide spanning the basic subdomain also recognizes TAR, identifying this subdomain as central for RNA interaction. TAR RNA forms

a stable hairpin that includes a six-residue loop, a trinucleotide pyrimidine bulge, and extensive duplex structure. Competition and interference expts. show that the Tat-derived fragments ***bind*** to ***double*** - ***stranded*** RNA and interact specifically at the pyrimidine bulge and adjacent duplex of TAR.

L9 ANSWER 68 OF 77 CA COPYRIGHT 1999 ACS
AN 114:18918 CA
TI Regulation of gene expression with ***double*** - ***stranded*** phosphorothioate oligonucleotides
AU Bielinska, Anna; Shivdasani, Ramesh A.; Zhang, Liquan; Nabel, Gary J.
CS Howard Hughes Med. Inst., Univ. Michigan, Ann Arbor, MI, 48109-0650, USA
SO Science (Washington, D. C., 1883-) (1990), 250(4983), 997-1000
CODEN: SCIEAS; ISSN: 0036-8075
DT Journal
LA English
AB Alteration of gene transcription by inhibition of specific transcriptional regulatory proteins is necessary for detg. how these factors participate in cellular differentiation. The functions of these proteins can be antagonized by several methods, each with specific limitations. Inhibition of sequence-specific DNA- ***binding*** proteins was achieved with ***double*** - ***stranded*** (ds) phosphorothioate oligonucleotides that contained octamer or .kappa.B consensus sequences. The phosphorothioate oligonucleotides specifically bound either octamer transcription factor or nuclear factor (NF)-.kappa.B. The modified oligonucleotides accumulated in cells more effectively than std. ds oligonucleotides and modulated gene expression in a specific manner. Octamer-dependent activation of a reporter plasmid or NF-.kappa.B-dependent activation of the human immunodeficiency virus (***HIV***) enhancer was inhibited when the appropriate phosphorothioate oligonucleotide was added to a transiently transfected B cell line. Addn. of phosphorothioate oligonucleotides that contained the octamer consensus to Jurkat T leukemia cells inhibited interleukin-2 (IL-2) secretion to a degree similar to that obsd. with a mutated octamer site in the IL-2 enhancer. The ***double*** - ***stranded*** phosphorothioate oligonucleotides probably compete for ***binding*** of specific transcription factors and may provide anti-viral, immunosuppressive, or other therapeutic effects.

L9 ANSWER 69 OF 77 CA COPYRIGHT 1999 ACS
AN 113:130529 CA
TI ***Binding*** of Tat protein to TAR region of human immunodeficiency virus type 1 blocks TAR-mediated activation of (2'-5')oligoadenylate synthetase
AU Schroeder, Heinz C.; Ugarkovic, Durdica; Wenger, Rosemarie; Reuter, Petra;

Okamoto, Takashi; Mueller, Werner E. G.
CS Inst. Physiol. Chem., Univ. Mainz, Mainz, 6500, Fed. Rep. Ger.
SO AIDS Res. Hum. Retroviruses (1990), 62(5), 659-72
CODEN: ARHRE7; ISSN: 0889-2229
DT Journal
LA English
AB The TAR sequence of the 5' leader of ***HIV*** -1 long terminal repeat-directed mRNA was found to be able to ***bind*** to and to activate ***double*** - ***stranded*** RNA-dependent (2'-5')A synthetase. ***Binding*** of TAR to the purified synthetase in vitro was abolished by addn. of ***HIV*** -1 Tat protein, which ***binds*** to this sequence with a high affinity. Inhibition of TAR-mediated activation of (2'-5')A synthetase by Tat was prevented in the presence of the Zn2+ and Cd2+ chelators o-phenanthroline and penicillamine, which did not impair TAR-synthetase interaction. Transient expression assays of bacterial chloramphenicol acetyltransferase (CAT) gene in HeLa cells revealed that the levels of both CAT mRNA and CAT protein decreased after treatment of the cells with interferon, if CAT gene was linked to ***HIV*** -1 TAR segment. Cotransfection of the cells with a tat sequence contg. plasmid rendered CAT gene expression insensible to the action of interferon.

L9 ANSWER 70 OF 77 CA COPYRIGHT 1999 ACS
AN 113:55597 CA
TI Phosphate-methylated DNA aimed at ***HIV*** -1 RNA loops and integrated DNA inhibits viral infectivity
AU Buck, Henk M.; Koole, Leo H.; Van Genderen, Marcel H. P.; Smit, Lia; Geelen, Jan L. M. C.; Jurriaans, Suzanne; Goudsmit, Jaap
CS Dep. Org. Chem., Eindhoven Univ. Technol., Eindhoven, 5600 MB, Neth.
SO Science (Washington, D. C., 1883-) (1990), 248(4952), 208-12
CODEN: SCIEAS; ISSN: 0036-8075
DT Journal
LA English
AB Phosphate-methylated DNA hybridizes strongly and specifically to natural DNA and RNA. Hybridization to single-stranded and ***double*** - ***stranded*** DNA leads to site-selective blocking of replication and transcription. Phosphate-methylated DNA was used to interrupt the life cycle of the human immunodeficiency virus type-1 (***HIV*** -1), the causative agent of acquired immunodeficiency syndrome (AIDS). Both antisense and sense phosphate-methylated DNA 20-nucleotide oligomers, targeted at the transactivator responsive region and the primer ***binding*** site, caused complete inhibition of viral infectivity at a low concn. Hybridization of phosphate-methylated DNA with folded and unfolded RNA was studied by UV and proton NMR spectroscopy. The combined results of hybridization studies and biol. expts. suggest that the design

of effective antisense phosphate-methylated DNA should focus on hairpin loop structures in the viral RNA. For sense systems, the 5' end of the integrated viral genome is considered to be the important target site.

L9 ANSWER 71 OF 77 CA COPYRIGHT 1999 ACS
AN 111:111875 CA
TI Temperature-gradient gel electrophoresis of nucleic acids: analysis of conformational transitions, sequence variations, and protein-nucleic acid interactions
AU Riesner, Detlev; Steger, Gerhard; Zimmat, Rolf; Owens, Robert A.; Wagenhoefer, Manfred; Hillen, Wolfgang; Vollbach, Silke; Henco, Karsten
CS Inst. Phys. Biol., Heinrich-Heine-Univ., Duesseldorf, D-4000, Fed. Rep. Ger.
SO Electrophoresis (Weinheim, Fed. Repub. Ger.) (1989), 10(5-6), 377-89
CODEN: ELCTDN; ISSN: 0173-0835
DT Journal
LA English
AB Temp.-gradient gel electrophoresis (TGGE) is applied for anal. of conformational transitions and sequence variations of nucleic acids and protein-nucleic acid interactions. A linear and highly reproducible temp.-gradient is established perpendicular or parallel to the direction of the electrophoresis. The instrument consists of an elec. insulated metal plate, which is heated at one edge and cooled at the other edge by two thermostating baths and is used as an ancillary device for com. horizontal gel electrophoresis instruments. Biopolymers are sepd. in TGGE according to size, shape, and thermal stability of their conformational transitions. If the temp.-gradient is established perpendicular to the electrophoresis, monomol. conformational transitions of nucleic acids show up as continuous transition curves; strand-sepn. leads to discontinuous transitions. In the studies on viroid RNA it was shown that natural circular viroid RNA undergoes one highly cooperative transition detected by TGGE as a drastic retardation in mobility. Oligomeric replication intermediates of viroids exhibit coexisting structures which could not be detected by any other technique. ***Double*** - ***stranded*** satellite RNA from cucumber mosaic virus is a mixt. of sequence variants, all of which have the identical length of 335 nucleotides. In TGGE six different strains were resolved. Sequence variants of viroids were analyzed by hybridizing viroid RNA to (-)strand viroid RNA transcripts from viroid cDNA clones. Sequence variations lead to mismatches in the ***double*** ***strands*** and thereby to a shift of the transition curve to lower temp. Mutations in plasmids, particularly in cloned inserts, were detected by mixing plasmids of two different clones, linearizing, denaturing, renaturing, and searching for shifts in the transition curves which are generated by mismatch-formation during the renaturation of (+)- and (-)-strands from different clones. Examples are

given for different viroid clones and ***HIV*** -clones from one and the same patient. In another example, clones with point mutations from site-directed mutagenesis are analyzed and selected by TGGE. TGGE is also applied to study the effect of amino acid exchanges in the Tet repressor from E. coli on the thermal stability of the repressor and on the mode of ***binding*** of the repressor to the operator DNA. The results are discussed under the aspect that TGGE may be applied as routine anal. lab. procedure.

L9 ANSWER 72 OF 77 CA COPYRIGHT 1999 ACS
AN 111:18806 CA
TI Yeast RNA vector for production of proteins such as vaccines
IN Wickner, R. B.; Fujimura, T.; Esteban, R.
PA United States Dept. of Health and Human Services, USA
SO U. S. Pat. Appl., 21 pp. Avail. NTIS Order No. PAT-6-168 486.
CODEN: XAXXAV
PI US 168486 A0 19880901
AI US 88-168486 19880317
DT Patent
LA English
AB A high-copy no. (>10,000/cell), stably maintain virus-like particle contg. RNA encoding a desired protein is prepd. from cDNA of a deletion mutant of the L-A ***double*** - ***stranded*** RNA of the yeast Saccharomyces cerevisiae into which is inserted an expression cassette for the desired protein. Yeast infected with the recombinant cDNA and the wt L-A genome produces modified virus-like particles which replicate and produce mRNA and the desired protein. A vector for ***HIV*** vaccine prodn. contg. the GAL1 upstream activating sequence and promoter, cDNA from the 5' end of L-A, the ***HIV*** gp120env gene, the cDNA for L-A protein ***binding*** site and the 3' end, the GAL1 terminator, a selectable yeast gene, and PUC18 DNA was prepd.

L9 ANSWER 73 OF 77 CA COPYRIGHT 1999 ACS
AN 110:169143 CA
TI Human immunodeficiency virus 1 reverse transcriptase. Template ***binding*** , processivity, strand displacement synthesis, and template switching
AU Huber, Hans E.; McCoy, John M.; Seehra, Jasbir S.; Richardson, Charles C.
CS Dep. Biol. Chem. Mol. Pharmacol., Harvard Med. Sch., Boston, MA, 02115, USA
SO J. Biol. Chem. (1989), 264(8), 4669-78
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
AB The kinetics of DNA synthesis catalyzed by reverse transcriptase from

human immunodeficiency virus 1 (***HIV*** -1) were analyzed. Reverse transcriptase, overproduced in Escherichia coli and purified to homogeneity, has polymerase and RNase H activity. Reverse transcriptase forms a stable complex with poly(rA).cntdot.oligo(dT) primer-templates in the absence of Mg2+ and dTTP with an equil. dissocn. const. of 3 nM. Synthesis from these preformed complexes can be initiated, and restricted to a single processive cycle, by the simultaneous addn. of Mg2+, dTTP, and excess competitor RNA. Preformed complexes decay with a maximal half-life of 2-3 min. Synthesis on poly(rA) templates is processive with an incorporation rate of 10-15 nucleotides/s at 37.degree.. Processivity varies widely with the template used, increasing from a few to >300 nucleotides in the order: poly(dA) < ***double*** - ***stranded*** DNA < single-stranded DNA < single-stranded RNA < poly(rA). On ***double*** - ***stranded*** DNA reverse transcriptase catalyzes limited strand-displacement synthesis of .ltoreq.50 nucleotides. On RNA-DNA hybrids significant DNA synthesis is obsd. only after degrdn. of the RNA strand by the RNase H activity of reverse transcriptase. Intermol. strand switching occurs with poly(rA) templates. At low ionic strength reverse transcriptase can use multiple templates with a single primer, leading to products of greater than template length. Reverse transcriptase and primer do not have to dissoc. during the exchange of template strands, thus allowing processive DNA synthesis across template borders.

L9 ANSWER 74 OF 77 CA COPYRIGHT 1999 ACS
AN 110:167011 CA
TI Inhibition of ***HIV*** -1 proviral DNA synthesis and RNA accumulation by mismatched dsRNA
AU Montefiori, David C.; Pellegrino, Michael G.; Robinson, W. Edward, Jr.; Engle, Karen; Field, Maggie; Mitchell, William M.; Gillespie, David H.
CS Dep. Pathol., Vanderbilt Univ., Nashville, TN, 37232, USA
SO Biochem. Biophys. Res. Commun. (1989), 158(3), 943-50
CODEN: BBRCA9; ISSN: 0006-291X
DT Journal
LA English
AB The antiviral activity of mismatched dsRNA of the form poly(I):poly(C12-U)n (Ampligen) against the human immunodeficiency virus type 1 (***HIV*** -1) was investigated by RNA-RNA and RNA-DNA hybridizations. Mismatched dsRNA delayed the appearance of newly transcribed ***HIV*** -1 RNA, as detected by liq. dot-blot hybridization in cultures of H9 T-lymphoblastoid cells following virus challenge. The appearance of proviral DNA as detected by Southern hybridization following virus challenge in H9 cells was also delayed. Mismatched dsRNA had no effect in syncytium inhibition assays performed by fusing MT-2 cells with H9/HTLV-IIIB cells. These results suggest that the

in vitro anti- ***HIV*** -1 activity of mismatched dsRNA occurs, at least in part, at an early stage in the viral replication cycle following initial gp120-CD4 ***binding*** .

L9 ANSWER 75 OF 77 CA COPYRIGHT 1999 ACS
AN 110:71372 CA
TI Intracellular localization and DNA- ***binding*** properties of human papillomavirus type 18 E6 protein expressed with a baculovirus vector
AU Grossman, Steven R.; Mora, Rene; Laimins, Laimonis A.
CS Howard Hughes Med. Inst., Univ. Chicago, Chicago, IL, 60637, USA
SO J. Virol. (1989), 63(1), 366-74
CODEN: JOVIAM; ISSN: 0022-538X
DT Journal
LA English
AB The E6 protein of human papillomavirus type 18 (***HPV*** -18) is a putative zinc-finger protein that is expressed in ***HPV*** -18-induced genital neoplasias. The biochem. properties of E6 protein synthesized in large amts. with a baculovirus expression vector were studied. When E6 protein was synthesized in insect cells infected with an E6-expressing baculovirus, the protein was localized to both nuclear and membrane fractions, with half-lives of 4 and 2 h, resp. Changing the first 5 amino acids of E6 did not alter the pattern of cellular localization of the protein but dramatically increased the half-life of the nuclear component to longer than 30 h and increased the half-life of the membrane component to 8 h. Although the baculovirus-expressed E6 protein bound to ***double*** - ***stranded*** DNA with high affinity, no sequence specificity for ***HPV*** -18 DNA was detected.

L9 ANSWER 76 OF 77 CA COPYRIGHT 1999 ACS
AN 108:92904 CA
TI Identification and characterization of ***HIV*** -specific RNase H by monoclonal antibody
AU Hansen, Jutta; Schulze, Thomas; Mellert, Werner; Moelling, Karin
CS Abt. Schuster, Max-Planck-Inst. Mol. Genet., Berlin, D-1000/33, Fed. Rep. Ger.
SO EMBO J. (1988), 7(1), 239-43
CODEN: EMJODG; ISSN: 0261-4189
DT Journal
LA English
AB Human immune deficiency virus (***HIV***) replicates by conversion of the RNA genome into the ***double*** - ***stranded*** DNA provirus. The reverse transcriptase is not the only enzymic function crucial in DNA-provirus synthesis. A viral-encoded RNase H activity which specifically degrades RNA in RNA-DNA hybrids has been shown to be essential as well. It is demonstrated here that the ***HIV*** -reverse

transcriptase which consists of a 2-polypeptide complex, p66 and p51, copurifies with an RNase H activity which exhibits properties of a processive exonuclease. Only the p66 mol., not p51, is active as polymerase as evidenced by activated gel anal. The p66 exhibits RNase H activity when pptd. as immune complex by a monoclonal antibody raised against a bacterially expressed C-terminal portion of p66. The monoclonal antibody which does not interfere with enzyme activity also ppts. a second population of mols. with RNase H activity which is of low mol. wt., p15. This RNase H appears therefore to be derived from the C-terminus of p66 during processing to the p51 polypeptide. It exhibits low template- ***binding*** ability and has a non-processing mode of action which may be due to the absence of the reverse transcriptase domain. These results lend exptl. support to the hypothesis that the RNase H gene maps at the C terminus of the reverse transcriptase.

L9 ANSWER 77 OF 77 CA COPYRIGHT 1999 ACS
AN 108:1317 CA
TI Expression and characterization of viral proteins from BPV-1, ***HPV*** -6b, and ***HPV*** -16
AU Mallon, R. G.; Wojciechowicz, D.; Defendi, V.
CS Kaplan Cancer Cent., New York Univ., New York, NY, 10016, USA
SO Cancer Cells (1987), 5(Papillomaviruses), 93-100
CODEN: CACEEG; ISSN: 0743-2194
DT Journal
LA English
AB Papillomavirus (PV) early proteins E2 [bovine papillomavirus type 1 (BPV-1) and human papillomarivus type 6b (***HPV*** -6b)] and E6 (BPV-1, ***HPV*** -6b, and ***HPV*** -16) bound ***double*** - ***stranded*** DNA. PV proteins were synthesized in Escherichia coli, each with a .lambda. cII gene 13-amino-acid leader sequence. The N-terminal 13 amino acids do not play a role in DNA ***binding*** . PV protein DNA ***binding*** was nonsequence-specific, under the assay conditions described here, and occurred between 50 and 200 mM NaCl at pH 7.0. Some PV proteins (e.g., BPV-1 E2) showed enhanced DNA ***binding*** at lower pH (pH 6.0) and NaCl concn. (50-100 mM). Antibodies generated to these early proteins inhibited DNA ***binding*** and identified the ***HPV*** -16 E6 protein in the SiHa human tumor cell line. It has been shown that the BPV-1 E2 open reading frame (ORF) encodes a trans-acting factor, and the DNA- ***binding*** activity demonstrated here suggests a mechanism for this action. DNA ***binding*** by E6 proteins reflects homol. (Cys-X-X-Cys repeats; a high percentage of Lys + Arg residues) found in other (SV40 large and small T and polyoma small T antigens) DNA- ***binding*** proteins.

(FILE 'HOME' ENTERED AT 19:33:25 ON 15 FEB 1999)

FILE 'CA' ENTERED AT 19:33:29 ON 15 FEB 1999

L1 120 S WEININGER?/AU
L2 3657 S TRIPLEX OR TRIPLE(W)HELI?
L3 0 S L1 AND L2
L4 60 S (HIV OR HPV) AND L2
L5 32 S L4 NOT (PY=1999 OR PY=1998 OR PY=1997 OR PY=1996)
L6 5345 S DOUBLE(W)STRAND? AND (BINDING OR BINDS OR BIND)
L7 0 S L1 AND L6
L8 124 S (HIV OR HPV) AND L6
L9 77 S L8 NOT (PY=1999 OR PY=1998 OR PY=1997 OR PY=1997 OR PY=1996)
L10 5 S THERAPEUTIC? AND L9